Available online at www.sciencedirect.com
SCIENCE DIRECT®
Environmental Research 100 (2006) 50–76
Environmental Research
www.elsevier.com/locate/envres

Large effects from small exposures. II. The importance of positive controls in low-dose research on bisphenol A

Frederick S. vom Saal[a,*], Wade V. Welshons[b]

[a]Division of Biological Sciences, University of Missouri-Columbia, Columbia, MO 65211, USA
[b]Department of Biomedical Sciences, University of Missouri-Columbia, Columbia, MO 65211, USA

Received 5 November 2004; received in revised form 24 August 2005; accepted 1 September 2005
Available online 27 October 2005

Abstract

Over six-billion pounds per year of the monomer bisphenol A (BPA) are used to manufacture polycarbonate plastic products, resins lining cans, dental sealants, and polyvinyl chloride plastic products. There are 109 published studies as of July 2005 that report significant effects of low doses of BPA in experimental animals, with many adverse effects occurring at blood levels in animals within and below average blood levels in humans; 40 studies report effects below the current reference dose of 50 μg/kg/day that is still assumed to be safe by the US-FDA and US-EPA in complete disregard of the published findings. The extensive list of significant findings from government-funded studies is compared to the 11 published studies that were funded by the chemical industry, 100% of which conclude that BPA causes no significant effects. We discuss the importance of appropriate controls in toxicological research and that positive controls are required to determine whether conclusions from experiments that report no significant effects are valid or false.

Keywords: Endocrine-disrupting chemicals; Environmental estrogens; DES; Bisphenol A; Positive controls; Low dose

1. Introduction

Bisphenol A (BPA) is an estrogenic endocrine-disrupting monomer used in the manufacture of polycarbonate plastic products, including many types of food and beverage containers. It is also the monomer used to manufacture the resin that lines metal food and beverage cans, and BPA is used as an additive in polyvinyl chloride and many other products such as flame retardants. BPA for many years has been one of the highest-volume chemicals in worldwide production, with annual production capacity in excess of six-billion pounds in 2003 and continued growth in production expected (Burridge, 2003). BPA is used in dental sealants, as is BPA dimethacrylate (BIS-DMA), which also has estrogenic activity and is metabolized in vivo to BPA (Darmani and Al-Hiyasat, 2004; Wada et al., 2004). The ester bond linking BPA molecules in polycarbonate and resins is subject to hydrolysis, resulting in leaching of BPA monomer even from new polycarbonate into water at room temperature (Howdeshell et al., 2003). The rate of leaching due to hydrolysis of the ester bonds (which link BPA molecules in polycarbonate and resins) increases as temperature increases and in response to acidic or basic conditions (Carey, 2003). The consequence is that, while polycarbonate is marketed as being highly durable, as polycarbonate begins to show signs of wear, the rate of leaching can increase over 1000-fold relative to the rate of leaching from new products (Factor, 1996; Howdeshell et al., 2003).

Sir Charles Edward Dodds reported in 1936 that BPA mimicked the activity of the hormone estradiol (Dodds and Lawson, 1936). BPA is similar in structure and efficacy to the estrogenic drug diethylstilbestrol (DES), which was synthesized by Dodds a few years after the initial report concerning BPA and other bisphenols that he was screening for potential use as estrogenic drugs (Dodds et al.,

*Corresponding author. Fax: +1 573 884 5020.
E-mail address: vomsaalf@missouri.edu (F.S. vom Saal).

0013-9351/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.envres.2005.09.001

1938). In 1952 when chemists first created polycarbonate from BPA, it was thus already clear that BPA was a chemical estrogen and that the use of BPA in products would result in leaching of free BPA leading to human exposure. It was not until 1999 that methods were described (Takao et al., 1999a) that allowed the detection of BPA at levels even close to the 1 pM (0.23 ppt) sensitivity of tissues to BPA (Wozniak et al., 2005), although even high-resolution GC-MS is still about 100-fold less sensitive than tissues to BPA, and many analytical studies report part per billion (ppb) detection limits.

It is inappropriate to use older studies that reported the absence of leaching of BPA from polycarbonate products based on very insensitive methods as the basis for claiming that there is little exposure of humans to BPA. A news article that discussed the new methods showing that there was significant leaching of BPA from baby bottles (ScienceNewsOnline, 1999) contrasted dramatically with comments by an FDA official, Dr. George Pauli, at the same time, who was quoted as stating that "with baby bottles, we haven't been able to detect BPA if we use reasonable extraction techniques." [Endocrine/Estrogen Letter, Vol. 5, No. 10 (106), 5/20/99]. There is now an extensive published literature documenting the rate of leaching of BPA from products and the significant part per billion concentrations of unconjugated BPA detected in human blood and tissues. A document containing references concerning BPA is posted on our web site (vom Saal, 2005a). As described below, this published literature is being ignored by the chemical industry and the US-FDA and US-EPA.

Until recently, BPA had been considered to be a very weak environmental estrogen, since in some bioassays (for example, the uterus of a number of rat and mouse strains or some responses in human MCF-7 breast cancer cells) BPA can be 10,000- to 100,000-fold less potent than estradiol, based on binding to receptors located in the cell nucleus (ERα and ERβ) (Welshons et al., 2003). For example, a common statement about BPA was that it "elicits weak estrogenic activity in in vitro and in vivo test systems" (Snyder et al., 2000). In contrast, since that statement was made, research has shown that the lowest-observed-effect concentration (LOEC) for BPA in MCF-7 cells is 100 pM (23 ppt) (Walsh et al., 2005) and in rat pituitary cells is 1 pM (0.23 ppt) (Wozniak et al., 2005). At a dose of 0.025 μg/kg/day administered tonically by Alzet pump to pregnant CD-1 mice, BPA causes abnormal growth of the mammary gland ducts (Markey et al., 2001a, 2003; Munoz-de-Toro et al., 2005) and altered postnatal growth, rate of sexual maturation, uterine function, and estrous cycles in female offspring (Markey et al., 2003, 2005). These studies in addition to over 100 other low-dose BPA studies with experimental animals published over the past 8 years and a large number of studies of cellular mechanisms have not had any apparent impact on the view of those associated with chemical corporations, who continue to state in publications that "Bisphenol A (BPA) is a weakly estrogenic monomer used in the production of polycarbonate plastic and epoxy resins" (Teeguarden et al., 2005). This statement contrasts dramatically with the conclusions drawn by those not associated with chemical corporations that "The ability of BPA to act as a highly potent E2-mimetic and to also disrupt the rapid actions of E2 at very low concentrations [1 pM; 0.23 ppt] during cerebellar development highlights the potential low-dose impact of xenoestrogens on the developing brain" (Zsarnovsky et al., 2005).

We will begin by briefly discussing the "low-dose" issue. This issue is covered in more detail in previous reviews (Welshons et al., 2003; vom Saal and Hughes, 2005). We will then discuss in some detail the published studies in which the authors have concluded that findings for low doses of BPA are not statistically significant. The major purpose of this review is to discuss the importance of control procedures in designing and analyzing research. We will discuss why it is impossible to interpret the results of the studies that report no significant effects of low doses of BPA in experiments in which there was either no positive control group or, in a few cases, a positive control group was included in the design but was misrepresented due to the fact that it did not show a difference relative to the negative control group. The purpose of a positive control group is to demonstrate that the test system is sensitive to the class of chemical being examined and to demonstrate competence to find a positive outcome for a chemical with demonstrated positive activity in the assay.

Since the published literature concerning in vivo effects and in vitro cellular mechanisms governing significant low-dose effects of BPA has recently been reviewed (vom Saal and Hughes, 2005), we have chosen to provide a table listing publications as of July 2005 rather than attempt to describe in any detail the rapidly growing literature. The document posted on our endocrine disruptors web site at the University of Missouri containing these and many other references concerning BPA is periodically updated and can be down-loaded (vom Saal, 2005a). Since this list of references is likely to be incomplete, we request that authors of omitted articles inform us so that we can include these in this periodically updated document.

2. The basis for our initial prediction of low-dose effects of BPA

Based on studies that we were conducting on the regulation of uptake of estrogenic chemicals from blood into tissues (Nagel et al., 1997) and on experimental evidence showing the exquisite sensitivity of fetal tissues to estrogenic chemicals (vom Saal et al., 1997), we predicted that BPA would have a much higher estrogenic potency in the fetal mouse than predicted based on studies conducted in the 1980s that used only very high doses ($\geqslant$50 mg/kg/day). Our prediction that BPA would cause effects at very low doses was based on the observation that a high proportion of BPA was free in blood (relative to estradiol) due to limited binding of BPA to plasma binding proteins

(Nagel et al., 1997). We presented detailed calculations that led us to predict that a 20-μg/kg/day dose of BPA fed to pregnant female mice would stimulate an increase in prostate size, decrease daily sperm production, and alter other reproductive organs in male offspring (Nagel et al., 1997; vom Saal et al., 1998), similar to effects observed due to maternal administration of very low (0.02–0.2 μg/kg/day) doses of both DES (vom Saal et al., 1997) and ethinylestradiol (Thayer et al., 2001), our positive control estrogenic chemicals. We had also shown that an increase of 0.1 pg/mL in free serum estradiol in male mouse fetuses (via a Silastic capsule implanted into pregnant mice) from the control level of 0.2 pg/mL to 0.3 pg/mL altered development of the urogenital system and permanently increased prostate size and prostatic androgen receptors (vom Saal et al., 1997).

Taken together, the above findings showed the exquisite sensitivity of the fetal urogenital system in male mice from an outbred stock (Charles River CF-1) to endogenous and exogenous estrogen. These initial low-dose positive control and subsequent BPA findings have been replicated in an independent study (Gupta, 2000a) and by us (Timms et al., 2005) in another outbred stock commonly used in toxicological studies, the Charles River CD-1 mouse, which was also used in the studies of effects of developmental exposure to very low doses of BPA on females by Markey and colleagues described above. The CD-1 mouse is the model animal used by the National Toxicology Program (NTP). Numerous studies conducted at the NTP with CD-1 mice have also confirmed a high sensitivity of this mouse stock to estrogenic chemicals when exposure occurs during critical periods in development (Newbold et al., 2001, 2004, 2005; Jefferson et al., 2005).

It is critical that an appropriate animal model be selected when examining chemicals whose mechanism of action has been determined. This was emphasized by a NTP panel (the Low-Dose Peer Review panel) that examined the issue of low-dose effects of endocrine-disrupting chemicals in animals. They stated in their report (available on the internet) that "Because of clear species and strain differences in sensitivity, animal model selection should be based on responsiveness to endocrine active agents of concern (i.e. *responsive to positive controls*), not on convenience and familiarity" (NTP, 2001, p. vii). In a previous review, the insensitivity of a commonly used animal model (the Charles River CD–Sprague–Dawley (CD-SD) rat, which is markedly different from the stock purchased from Sprague–Dawley 50 years ago) to any exogenous estrogen, including potent estrogenic drugs, was discussed in relation to many often-cited studies reporting no significant effects of low doses of BPA (vom Saal and Hughes, 2005). It was a study conducted with this rat model (Tyl et al., 2002) that reported no significant effects of low doses of BPA (but did not include a positive control) that prompted the above statement from the NTP Low-Dose Peer Review panel. After the NTP report, the US-EPA contracted to have the issue of variability in strains of experimental animals used in toxicological research reviewed in a "white paper." Unfortunately, this document has generated more controversy than enlightenment, and the US-EPA recently posted a critique written by Dr. Jimmy Spearow along with the original white paper on the internet at http://www.epa.gov/scipoly/oscpendo/program/whitepaper.htm.

3. Low-dose BPA findings challenge the assumptions used in chemical risk assessments

Our finding that in utero exposure to very low maternal doses of BPA (2–20 μg/kg/day) exerted estrogenic effects in male and female mice initiated a controversy referred to as the "low-dose" issue in toxicology. Specifically, our findings that a 2–10-μg/kg/day dose of BPA could alter fetal development, leading to permanent changes in reproductive function and behavior (Nagel et al., 1997; vom Saal et al., 1998; Howdeshell et al., 1999; Palanza et al., 2002; Timms et al., 2005) and a large number of other low-dose findings (see Table 1) contrast with the estimate of the "safe" human exposure level for BPA calculated from very high-dose studies in which the lowest dose of BPA tested was 50 mg/kg/day; this very high dose is still considered to be the lowest-observed-adverse-effect level (LOAEL) by the US-EPA, since adverse effects were reported at this dose. The US-EPA used the LOAEL of 50 mg/ kg/day to estimate a reference dose for BPA of 50 μg/kg/day by applying a 1000-fold safety factor (IRIS, 1988). However, no attempt to actually determine in an experiment whether this estimate of the safe human exposure level was actually far below the no-observed-effect level (NOEL) was ever made prior to our initial study (Nagel et al., 1997).

An important aspect of recent studies on the effects of BPA listed in Table 1 is that they represent a sharp departure from traditional toxicological studies with regard to dose selection (Calabrese and Baldwin, 1997; vom Saal and Sheehan, 1998). The focus of current research on BPA and other endocrine-disrupting chemicals is on doses within an "environmentally relevant range," that is, doses within the range of exposure of wildlife and humans. This range is known for chemicals such as BPA for which there are substantial human exposure data from different countries (vom Saal, 2005a). This new "low-dose range" is typically many orders of magnitude lower than doses used in toxicological studies conducted for regulatory purposes, where a few doses in the "toxicological dose range," typically within 50-fold of the maximum tolerated dose (MTD), are studied (vom Saal and Sheehan, 1998; Welshons et al., 1999). "Low dose" as now used by regulatory agencies, based on recommendations by the NTP's Low-Dose Peer Review Panel, thus refers to doses that are lower than the very limited range of doses typically used in toxicological studies and includes doses below the no-observed-adverse-effect level (NOAEL) or the LOAEL, based on studies that examined only a few very high doses (NTP, 2001).

Table 1
Published papers reporting biological effects in animal studies for BPA in the low-dose exposure range

Low-dose BPA studies in laboratory rats and mice (and one study each in sheep and gerbils) (<50,000 μg/kg BW/day; <50 mg/kg BW/day)

Authors (years)	Animal	Sex	Endpoints	Exposure, vehicle	Doses tested, μg/kg BW/day (*$P<0.05$)	Other chemicals tested
Nagel et al. (1997)	Mouse	Males	Prostate Wt.	Oral, oil	2*, 20*	Octylphenol
Colerangle and Roy (1997)	Rat	Females	Mammary gland	Implant	100*, 54,000*	DES
Steinmetz et al. (1997)	Rat	Females	Serum prolactin; PRF activity (see also in vitro)	Implant	300*	Estradiol
Gould et al. (1998)	Rat	Females	Uterine progesterone receptors	Oral, oil	5000*, 10,000*, 25,000*, 50,000*, 100,000*, 150,000*	Estradiol
			Uterine peroxidase		5000*, 10,000, 25,000, 50,000, 100,000*, 150,000*	
Steinmetz et al. (1998)	Rat	Females	Uterine epithelial height, vaginal epith. morphology	Implant	300*	Estradiol
vom Saal et al. (1998)	Mouse	Males	Reproductive organs	Oral, oil	2*, 20*	Octylphenol
			Sperm production		2, 20*	
Farabollini et al. (1999)	Rat	Females/males	Exploratory behavior, activity	Oral, oil	40*, 400*	
Fisher et al. (1999)	Rat	Males	Efferent duct epithelial height	Injection, oil	37,000*	DES, Ethinylestradiol, Genistein, Octylphenol, Parabens
Howdeshell et al. (1999)	Mouse	Females/males	Puberty, BW	Oral, oil	2.4*	
Takao et al. (1999b)	Mouse	Males	Plasma free testosterone	Oral, drinking water	≈120, ≈12,000*	
Elswick et al. (2000)	Rat	Males	Prostate Wt.	Oral, water	1, 10*, 100, 1000*, 10,000*	
Goloubkova et al. (2000)	Rat	Females	Uterine weight (uterotrophic response)	Injection, s.c.	11,000* to 250,000*	
Gupta (2000a)	Mouse	Males	Prostate Wt., prostate AR (see also in vitro)	Oral, oil	50*	DES, Aroclor
Khurana et al. (2000)	Rat	Females/males	Hyperprolactinemia	Injection, oil	15,000*, 75,000*	DES, Octylphenol
			Pituitary estrogen receptor alpha, beta expression		15,000*	
Long et al. (2000)	Rat	Females	F344 rat uterine BrdU incorporation	Injection, oil	200, 19,000, 37,500*, 75,000*, 150,000*	Estradiol
Talsness et al. (2000)	Rat	Females/males	Reproductive system effects	Oral, oil	100*, 50,000*	Ethinylestradiol
			Body weight, estrus cycle; AGD in males		100*, 50,000	
					100*, 50,000	
Aloisi et al. (2001)	Rat	Females	Estrogen receptor alpha	Oral, oil	40,000*	
Berg et al. (2001)	Bird	Females/males	Quail, chicken embryo abnormalities, ovotestes	Injection, into egg	≈4000*	
			Chicken embryo mortality		≈1340*, ≈4000*	
Funabashi et al. (2001)	Rat	Females	Hypothalamic preoptic area	Injection, s.c.	10,000*	Estradiol, Butyl benzyl phthalate
			Progesterone receptors			
Kubo et al. (2001)	Rat	Females/males	Brain, behavior	Oral, water	1500*	
Markey et al. (2001a)	Mouse	Females	Mammary gland	Osmotic minipump	25*, 250*	

Table 1 (*continued*)

Low-dose BPA studies in laboratory rats and mice (and one study each in sheep and gerbils) (<50,000 μg/kg BW/day; <50 mg/kg BW/day)

Authors (years)	Animal	Sex	Endpoints	Exposure, vehicle	Doses tested, μg/kg BW/day (*$P<0.05$)	Other chemicals tested
Markey et al. (2001b)	Mouse	Females	Uterine epithelial cell height	Osmotic minipump	100, 500, 1000, 5000*, 50,000, 75,000*, 100,000*	Estradiol
			Earlier vaginal opening		100*, 500, 1000, 5000, 50,000, 75,000, 100,000*	
Nagel et al. (2001)	Mouse	Females	Uterus, gene expression	Injection, oil	25 ($P<0.06$), 791*, 25,000*	DES
Nunez et al. (2001)	Rat	Female	Body weight gain, feeding efficiency	Osmotic minipump	4,500, 18,000*, 22,700*	
Ramos et al. (2001)	Rat	Males	Ventral prostate	Implant	25*, 250*	
Rubin et al. (2001)	Rat	Females	Body Wt., cyclicity, LH	Oral, water	100*, 1200*	
Sakaue et al. (2001)	Rat	Males	Sperm production	Oral, oil	0.2, 2, 20*, 200*, 2000*, 200,000*	
Tohei et al. (2001)	Rat	Males	Testes & serum hormones	Injection	3000*	
Al–Hiyasat et al. (2002)	Mouse	Males	Fertility, sperm count	Oral, water	5*, 25*, 100*	
Aloisi et al. (2002)	Rat	Females/males	Pain behavior	Oral, oil	40*	
Dessi-Fulgheri et al. (2002)	Rat	Females/males	Play behaviors	Oral, oil	40*, 400*	
					40*, 400	
					40, 400*	
Facciolo et al. (2002)	Rat	Females	$GABA_A$ receptors	Oral, oil	40, 400*	
Farabollini et al. (2002)	Rat	Females/males	Aggression, sexual behavior	Oral, oil	40*	
Honma et al. (2002)	Mouse	Females/males	BW, estrous cycle length, male AGD	Injection, oil	2*, 20*	DES
			Vaginal cytology; female AGD		2*, 20	
			Age of vaginal opening, first estrus		2, 20*	
Palanza et al. (2002)	Mouse	Females	Maternal behaviors	Oral, oil	10*	
Papaconstantinou et al. (2002)	Mouse	Females	Heat shock protein grp94 (others)	Injection, oil	1000*, 10,000*, 40,000*, 100,000*, 400,000*	Estradiol
Schönfelder et al. (2002b)	Rat	Females	Vaginal ER alpha expression	Oral	100*, 50,000*	Ethinylestradiol
Suzuki et al., (2002)	Mouse	Females	Uterine and vaginal mitotic indices following prenatal exposure	Injection, oil	10,000*, 100,000*	DES
Adriani et al. (2003)	Rat	Females/males	Behavioral tests, M & F; amphetamine response, M	Oral, oil	40*	
Carr et al. (2003)	Rat	Females/males	Morris water maze	Oral, oil	100, 250*	Estradiol
			Elimination of sex difference		100*, 250	
Chitra et al. (2003)	Rat	Males	Decreased testis, epididymis weight	Oral, oil	0.2*, 2*, 20*	
			Increased ventral prostate weight		0.2*, 2*, 20*	
			Reduced sperm motility		0.2*, 2*, 20*	
			Sperm count		0.2, 2*, 20*	
			Oxidative stress enzymes		0.2*, 2*, 20*	
			Increased H_2O_2		0.2*, 2*, 20*	
Funabashi et al. (2003)	Rat	Females	Hypothalamic progesterone receptors, POA and VMH	Oral, oil	4, 40, 400*, 4000*	Estradiol
Hunt et al. (2003)	Mouse	Females	Disruption of meiosis; aneuploidy	Oral, oil	20*, 40*, 100*	
Imanishi et al. (2003)	Mouse	Females/males	Placental nuclear receptor gene expression, 9 of 20 examined;	Oral, oil	2*	
			Six nonnuclear receptor genes		2*	

Kawai et al. (2003)	Mouse	Males	Aggression, testis Wt.	Oral, oil	2*, 20*	
Kabuto et al. (2003)	Mouse	Males	Oxidation enzymes	Injection, aqueous	25,000*, 50,000*	
Kubo et al. (2003)	Rat	Females/males	Brain/behavior	Oral	30*, 300*	DES, Resveratrol
Markey et al. (2003)	Mouse	Females	Estrus cycle alterations	Osmotic minipump	25*, 250*	
			Blood-filled ovarian bursae		25*, 250*	
			Mammary gland budding		25*, 250*	
Negishi et al. (2003)	Rat	Males	Behavioral alterations, males 8 weeks	Oral, oil	4000*, 40,000, 400,000	
Nishizawa et al. (2003)	Mouse	Females/males	Embryonic brain and gonad retinoid receptor expression RAR alpha, RXR alpha	Oral, oil	2*	
Ramos et al. (2003)	Rat	Males	Ventral prostate; HPG axis	Osmotic minipump	25*, 250*	
			Estrogen receptor beta		25*, 250*	
Sawai et al. (2003)	Mouse	Female	Decreased IFN gamma and IL-10 secretion by splenic mononuclear cells; decreased IgG2a production; protection in lupus-prone mice (see also in vitro)	Oral, in feed	2.5*	
Sugita-Konishi et al. (2003)	Mouse	Female	Immune cells and functions, reduced immunodefense against bacterial infection	Injection, oil	5000*	
					5000*	
Suzuki et al., (2003)	Mouse	Females	Dopamine D1 receptor-mediated enhanced induced abuse state	Oral, in feed	0.002, 0.5, 2 mg/g feed	
					≈300*, ≈75,000*, ≈300,000*	
Takahashi and Oishi (2003)	Rat, mouse	Males	BPA low-dose challenge after extended high-dose exposure	Injection, propylene glycol	2000, 20,000*	
Takao et al. (2003)	Mouse	Males	Testicular ER alpha, ER beta	Oral, drinking water	≈200, ≈20,000*	
Thuillier et al. (2003)	Rat	Males	Neonatal testicular gonocyte PDGF alpha, PDGF beta		100, 1000*, 10,000*, 200,000*	DES Genistein Coumestrol
Wistuba et al. (2003)	Rat	Males	Sertoli cell number per testis	Oral, cornstarch suspension	100*, 50,000*	Ethinylestradiol
Yoshino et al. (2003)	Mouse	Females/males	Antigen-specific antibody production; augmentation of immune response	Oral, oil	3, 30, 300*, 3000*	Estradiol
					3, 30*, 300*, 3000*	
Aikawa et al. (2004)	Mouse	Males	Abnormal sperm	Injection, oil	≈175*, ≈17,500*	Estradiol
			Decreased motility		≈175, ≈17,500*	
Akingbemi et al. (2004)	Rat	Males	Serum LH, testosterone, suppression	Oral, oil	2.4*, 10(*), 100,000, 200,000	
			Serum estradiol suppression		2.4*, 10*, 100,000*, 200,000	
			LH beta and ER alpha expression; body weight; seminal vesicle weight (see also in vitro)		2.4*	
Al-Hiyasat et al. (2004)	Mouse	Females	Increased resorptions, uterine weight	Oral, water	5, 25*, 100*	
			Ovarian weight		5, 25, 100*	
Darmani and Al-Hiyasat (2004)	Mouse	Females/males	Reproduction/fertility	Oral, aqueous (as BPA-DMA)	5*, 25*, 100*	

Table 1 (*continued*)

Low-dose BPA studies in laboratory rats and mice (and one study each in sheep and gerbils) (<50,000 μg/kg BW/day; <50 mg/kg BW/day)

Authors (years)	Animal	Sex	Endpoints	Exposure, vehicle	Doses tested, μg/kg BW/day (*$P<0.05$)	Other chemicals tested
			Resorptions–implantations		5*, 25*, 100	
			Body weight		5*, 25*, 100*	
			Sperm count & daily sperm production		5, 25*, 100* (as bisphenol A dimethacrylate)	
Evans et al. (2004)	Sheep	Females	Tonic LH secretion; LH pulse frequency & amplitude	Injection, oil	3500*	DES
						Octylphenol
Funabashi et al. (2004)	Rat	Females	Sex differences in CRH neurons in bed nucleus of the stria terminalis	Oral, drinking water	≈2000*	
Ishido et al. (2004)	Rat	Males	Hyperactivity	Intracisternal injection, oil 10 μl	≈3, ≈30, ≈300*, ≈3000*	
			Dopamine receptor and dopamine transporter		≈3, ≈30, ≈300, ≈3000*	
Kabuto et al. (2004)	Mouse	Males	Testis weight	Oral, drinking water	≈750*, ≈1500*	
			Brain weight		≈750*, ≈1500	
			Kidney weight		≈750, ≈1500*	
			Various oxidation markers		≈750*, ≈≈1500*	
Lemmen et al. (2004)	Mouse	Females/males	Transgenic embryonic gene expression in whole embryo lysates	Injection, oil	100, 1000*, 10,000*	DES, Estradiol
						Propionate
Masuo et al. (2004)	Rat		Motor hyperactivity (spontaneous motor activity)	Intracisternal injection, oil 10 μl	0.087, 0.87*, 8.7*, 87* nM	Nonylphenol, Octylphenol, DEHP
			Midbrain gene expression		87* nM	
Negishi et al. (2004)	Rat	Males	Behavioral alterations active avoidance test	Oral, oil	100*	Nonylphenol
Nikaido et al. (2004)	Mouse	Females	Cycle length, diestrus	Injection, DMSO	500*, 10,000*	DES, Genistein, Zearalenone, Resveratrol
			Reduced corpora lutea		500*, 10,000*	
			Mammary gland dev.		500*, 10,000*	
			Age at vaginal opening		500, 10,000*	
Schönfelder et al. (2004)	Rat	Females	Uterine epithelial histology	Oral	100*, 50,000*	Ethinylestradiol
			Uterine ER alpha, ER beta expression		100*, 50,000*	
Toyama and Yuasa (2004)	Mouse, rat	Males	Spermatogenesis, mouse	Injection, oil	71, 714*, 3,600*, 7,100*	Estradiol
			Rat		180, 1800*, 18,000*	Estradiol Benzoate
Wang et al. (2004)	Rat	Males	Estrogen receptor-associated protein expr: Hsp90	Oral, oil	1000, 10,000*, 200,000*	DES, Genistein, Coumestrol
			p23		1000*, 10,000, 200,000	
Yoshino et al. (2004)	Mouse	Females/males	IgG2a	Oral, oil	3, 30*, 300*, 3000*	
			T-helper cytokines Th1, Th2		3, 30, 300*, 3000*	
Zoeller and Rovet, 2004	Rat	Females/males	Serum thyroxin; dentate gyrus RC3/neurogranin expression	Oral, feed	1000*, 10,000*, 50,000*	

Della Seta et al. (2005)	Rat	Females	Maternal behavior	Oral, oil	40*	
Laviolaa et al. (2005)	Mouse	Females/males	Amphetamine-induced conditioned place preference	Oral, oil	10*	Methoxychlor
MacLusky et al. (2005)	Rat	Female	Hippocampal pyramidal neuron synaptogenesis	Injection, oil	40*, 120*, 400*	17 beta estradiol, 17 alpha estradiol
					45*	
Markey et al. (2005)	Mouse	Females	Genital tract alterations; estrogen receptor alpha and progesterone receptor up-regulation developmentally	Osmotic minipump	0.025*, 0.250*	
Munoz-de-Toro et al. (2005)	Mouse	Females	Mammary gland morphogenesis, terminal end bud density	Osmotic minipump	0.025*, 0.250*	
Nishizawa et al. (2005)	Mouse	Females/males	Embryonic brain and gonad aryl hydride receptor expression; RAR alpha, RXR alpha expression	Oral, oil	0.02*, 2*, 200*, 2000*	
Porrini et al. (2005)	Rat	Females/males	Socio-sexual behaviors	Oral, oil	40*	
Razzoli et al. (2005)	Gerbil	Females	Social investigative behavior	Oral, oil	2*, 20	Ethinylestradiol
			Free exploratory tests		2*, 20*; 2*, 20; & 2, 20*	
Timms et al. (2005)	Mouse	Males	Fetal development of prostate and urethra	Oral, oil	10*	Ethinylestradiol
Zoeller et al. (2005)	Rat	Females Males/females Males	Pregnancy body weight gain Day 15 pup serum T_4, and Male pup thyroid hormone-responsive RC3/neurogranin expression in brain	Oral, edible wafer	1000*, 10,000*, 50,000* 1000*, 10,000*, 50,000*	

Low-dose BPA studies in aquatic animals (< 1 ppm in water (ppb range); < μg/mL; < 1000 ng/mL; < 1000 μg/liter; < 4.39 μM)

Authors (years)	Animal	Sex	Endpoints	Exposure, vehicle	Exposure, ng/mL (ppb) (*$P < 0.05$)	Other chemicals tested
Kloas et al. (1999)	Frog	Females/males	Altered sex determination	Fresh water	2.3, 23*	Estradiol, Nonylphenol
Haubruge et al. (2000)	Guppy	Males	Total stored sperm count	Fresh water	274*, 549*	Tributyltin
Lindholst et al. (2000)	Fish		Vitellogenin synthesis	Fresh water	500*	
Oehlmann et al. (2000)	Snail	Females/males	Reproductive organs fertility	Marine, fresh water	1*, 5*, 25*,100*	Octylphenol
Arukwe et al. (2000)	Fish	Females/males	Eggshell zona radiata proteins, vitellogenin	Injection, i.p.	5000*	
Shioda and Wakabayashi (2000)	Fish	Males	Reduced fertility	Marine	2300*	Estradiol, Nonylphenol, DEHP
Kwak et al. (2001)	Fish	Males	Swordtail sword growth	Fresh water	0.2, 2*, 20*	Nonylphenol
Metcalfe et al. (2001)	Fish	Males	Sperm count	Fresh water	10, 50*, 100*, 200*	Estradiol, Estrone, Estriol, Nonylphenol

Table 1 (*continued*)

Low-dose BPA studies in aquatic animals (< 1 ppm in water (ppb range); < μg/mL; <1000 ng/mL; <1000 μg/liter; <4.39 μM)

Authors (years)	Animal	Sex	Endpoints	Exposure, vehicle	Exposure, ng/mL (ppb) (*$P<0.05$)	Other chemicals tested
Schulte-Oehlmann et al. (2001)	Snail	Females/males	Reproductive organs, "Superfemales"; LOEC number of embryos	Marine, fresh water	0.048*–100*	
					0.048*	
					5*, 25*, 100	
Sohoni et al. (2001)	Fish	Females/males	Reduced fertility		1*, 16*, 160*, 640*, 1280*	
			Reproductive organs		1*, 16*, 160*, 640, 1280	
			Spermatocyte male sex cell type			
Tabata et al. (2001)	Fish	Males	Female proteins,	Marine	10*	Estradiol, Nonylphenol
			Abnormal gonads		100*	
Watts et al. (2001)	Insect	Females/males *Chironomus*	Delay in emergence times of adult stage	Sediment spiking	<10, 0.078*, 0.55*, 77*, 750*	Ethinylestradiol
Hahn et al. (2002)	Insect	Females/males *Chironomus*	Vitellogenin, emerged males	Sediment spiking	1*, 100*, 3000*	Nonylphenol
			Females		1, 100, 3000*	
Kashiwada et al. (2002)	Fish	Males	Female specific proteins in male serum	Fresh water	0.1, 10*, 100*	Estradiol, Nonylphenol
Duft et al. (2003)	Snail	Females	Embryo production LOEC	Sediment spiking	1*	Nonylphenol, Octylphenol
			EC10, EC50		0.22*, 24.5*	
Jobling et al. (2003)	Snail	Females	Embryo production	Fresh water	1*, 6*, 25*, 100	Ethinylestradiol, Octylphenol
Tabata et al. (2003)	Fish	Males	Serum vitellogenin, 3 days to 5 weeks	Fresh water	100, 200, 500*, 1000*	Chlorinated BPAs
			3 weeks only		200*	
Van den Belt et al. (2003)	Fish	Male	Vitellogenin	Fresh water	40, 200, 1000*	Estradiol, Nonylphenol, Dibutylphthalate
Watts et al. (2003)	Insect	Females/males *Chironomus*	Larval mouthpart structure	Fresh water	0.01*, 0.1, 1*, 10, 100, 1000	Ethinylestradiol
			Molting		0.01, 0.1, 1, 10, 100, 1000*	
Honkanen et al. (2004)	Fish	Females/males	Liver histology (vacuoles, other)	Fresh water	10, 100*, 1000*	
Levy et al. (2004)	Frog	Females/males	Sex ratio	Fresh water	2.3, 23*, 230	Estradiol
			ER alpha mRNA up-regulation		23*	
Canesi et al. (2005)	Mussel	Females/males	Lysosomal membrane destabilization	Injection	5.7* ng/mL in hemolymph	
			MAPK and STAT			
			CREB-like transcription factor (see also in vitro)			
Trudeau et al. (2005)	Frog	Females/males	ERE-TK-LUC activity in transfected *Xenopus* tadpole brain	Fresh water	11.4*	

There have been a number of studies that have examined only very high doses of BPA that are above this new "low-dose" range. However, this review will focus only on "low-dose" studies. Unfortunately, the term "low dose" has been used in many studies in which all doses are within a very high toxicological dose range, and the lowest dose used is then referred to as a "low dose." If the standards for determining the "low-dose range" established by the NTP Low-Dose Peer Review panel (NTP, 2001) are followed, this type of confusion can be avoided.

There are 40 published in vivo studies showing a wide range of adverse effects of BPA at and below doses of 50 μg/kg/day, demonstrating that the assumptions used in the risk assessment process to calculate this presumably "safe" dose for human daily consumption are false (vom Saal, 2005a). These studies and the implications of such a large number of published studies showing adverse effects below the reference dose are covered in more detail in other publications (vom Saal and Sheehan, 1998; Welshons et al., 2003; vom Saal and Hughes, 2005; vom Saal et al., 2005). Thus, while our findings are of particular concern to BPA manufacturers, the "low-dose issue" is viewed as a threat by the entire chemical industry, which is faced with the prospect that acknowledgment of these findings will lead to the requirement to reestablish the reference dose for all chemicals in commerce based on new approaches to establishing acceptable levels of exposure to chemicals by testing a much wider range of doses than is the current practice. Our view is that the extensive low-dose BPA literature (and information about low-dose effects of other chemicals) clearly demonstrates the need to directly test low, environmentally relevant doses of chemicals in commerce for which there are exposure data, since extrapolation based on testing only very high doses failed to predict that BPA would be found to be a hazard at current human exposure levels in a large number of studies (Table 1). Even if there are no exposure data for a chemical, a much wider range of doses needs to be examined relative to the very few doses and narrow dose range currently required by regulatory agencies.

We are aware of 130 articles as of July 2005 published in peer-reviewed scientific journals that involved administration to animals of "low doses" of BPA, and many of these are listed in Table 1. Here we define "low dose" as a dose below 50 mg/kg/day, since this was used to calculate the current reference dose, and any confirmed findings of adverse effects below 50 mg/kg/day would result in a recalculation of the reference dose, even if the same flawed assumptions currently used in chemical risk assessments remain in place. There are additional in vivo studies and in vitro studies of the molecular mechanisms mediating BPA effects in cells and research on rates of leaching from various products, levels measured in food, drinking water, waterways, air, and soil, levels in human and other animal blood and tissue, and pharmacokinetics that are listed in the BPA document on our web site (vom Saal, 2005a).

Even though there is now an extensive low-dose published literature on BPA, there is still some confusion concerning what the no-effect dose is for BPA. An interesting discussion of the reason for stating in publications that they had concluded that the NOEL for BPA was 50 mg/kg/day (Society-of-the-Plastics-Industry, 1996, p. 24) was published by the Society of the Plastics Industry (SPI), an industry trade/lobbying organization. SPI stated about BPA that "Effects observed in the presence of maternal toxicity include fetal toxicity and decreased sperm motility, weight of testis, epididymis, and seminal vesicles. Because all effects were seen in the presence of maternal toxicity, BPA should not be considered a selective reproductive or developmental toxicant" (Society-of-the-Plastics-Industry, 1995, p. 7). This is a remarkable conclusion to reach for a chemical that was documented to be a full estrogen agonist in 1936 (Dodds and Lawson, 1936). Based on this finding 70 years ago, BPA would be expected to selectively interfere with the functioning of reproductive organs in males. The SPI went on to say that "The lowest-observed-effect Level (LOEL) was 50 mg/kg/day. Although a NOEL for these effects was not established in this study, the NOEL is probably not far below the LOEL of 50 mg/kg/day" (Society-of-the-Plastics-Industry, 1995, p. 21). This "leap of faith" led to the conclusion by the SPI that 50 mg/kg/day was, in fact, the no-observed-adverse-effect dose (Society-of-the-Plastics-Industry, 1995, p. 24). In contrast, the US-EPA identified 50 mg/kg/day as the LOEL for BPA and thus applied a 1000-fold safety factor to the LOEL to predict that exposure to 50 μg/kg/day BPA would be a "safe" daily exposure level for humans (IRIS, 1988).

The published studies listed in Table 1 reveal that a 50-mg/kg/day dose of BPA is 2,000,000 times higher than the 25-ng/kg/day dose reported to produce adverse effects (such as stimulation of mammary gland ductal development) in female mice (Markey et al., 2001a). The reported no-observed-effect concentration (NOEC) of BPA is 7.9 ppt in snails (Schulte-Oehlmann et al., 2001) and 5 ppt in the fetal mouse prostate in primary culture (Gupta, 2000a). Also, BPA stimulates calcium influx (followed by prolactin secretion) within 1 min in rat pituitary tumor cells at the lowest dose tested, which was 0.23 ppt (Wozniak et al., 2005). These findings provide strong evidence that BPA is far more potent than predicted by the traditional high-dose toxicological testing paradigm.

The European Union released a risk assessment of BPA in 2003 (ECB, 2003), but the comprehensive literature search was reported as having been conducted in 1998, at which time there were only five published studies of the effects of low doses of BPA in experimental animals, and only selected additional articles were examined through 2001 in this report. This EU document estimated that the NOAEL for BPA was 5 mg/kg/day. The EU report and the much older US-EPA risk assessment from the 1980s (IRIS, 1988) are now rendered obsolete by the very large number

of recently published studies showing adverse effects of low doses of BPA far below 5 mg/kg/day (Table 1).

The large number of findings that endocrine-disrupting chemicals such as BPA could have effects far below the presumed reference dose leads us to propose that regulatory agencies should actually require predicted safe doses to be tested for effects. In contrast, the current practice is to assume that the risk assessment threshold model for systemic toxicants (which BPA is considered) is valid and thus does not need to be verified by actual experimentation. In complete disregard of these published findings, the US-EPA still refuses to consider testing a much wider range of doses in studies conducted for risk assessment purposes (OMB-Watch, 2005), which are typically funded by the manufacturer (the significance of this will be discussed below).

Two basic assumptions in risk assessment are falsified by a very large number of studies with just low doses of BPA (there is obviously a much larger literature if other chemicals are also taken into consideration). First, the no-adverse-effect level is assumed to be 10-fold lower than the LOAEL, which provides the rationale for adding a 10-fold safety factor to the calculation of the reference dose when adverse effects are found at the lowest dose tested (as was the case for BPA). Second, testing very high doses to predict effects at very low doses would require all dose–response curves to be monotonic, which is an assumption that provides the basis for the extrapolation procedure using safety factors applied to results obtained from testing only very high doses used in the risk assessment process. In sharp contrast, it is well established in endocrinology that high doses of hormones and hormonally active drugs and chemicals can exert inhibitory effects on processes that are stimulated at much lower doses, resulting in inverted-U dose–response curves (Welshons et al., 2003). The issue of the lack of a threshold in a situation when the background level of the hormone being mimicked by an environmental chemical such as BPA already exceeds the threshold for response is discussed in more detail elsewhere (vom Saal and Sheehan, 1998; Sheehan et al., 1999; Welshons et al., 2003).

The response by chemical industry trade organizations has been to reject the possibility of nonmonotonic dose–response curves and that effects could occur at low doses that would not be predicted by experiments that examined only a few very high doses. For example, the Association of Plastics Manufacturers in Europe (APM) stated that "The fundamental principle of toxicology assumes that biological effects increase as the dose increases" (APM, 2005) [see also the response to this commentary (vom Saal, 2005b)]. In contrast, there are 16 published studies showing inverted-U dose–response curves just for BPA and examples for other types of chemicals. Of course, these findings required examination of a wide range of doses, including doses far below those that would have been tested in a traditional high-dose toxicological study conducted for regulatory purposes, which, of course, could never reveal an inverted-U dose–response relationship.

One example of an inverted-U dose–response curve from an experiment concerning molecular mechanisms mediating low-dose effects of BPA was reported in a study using rat pituitary tumor cells. In these cells and in a number of other cell types, BPA rapidly stimulated calcium influx (followed by prolactin secretion) within 1 min via receptors associated with the cell membrane that have, as yet, not been identified. BPA stimulated calcium influx at the lowest dose tested, which was 0.23 ppt BPA, and the maximum response occurred at 230 ppt BPA, but at 2300 ppt BPA the response declined by about 50% relative to the response at 230 ppt BPA (Wozniak et al., 2005). The plastic manufacturers misrepresent this type of finding. For example, the APM commented that "The low-dose hypothesis asserts that health effects may be observed at extremely low doses, while higher doses do not have any effects." This is misleading in that it is well recognized by anyone familiar with the endocrine literature that, as the dose of a hormone (or hormone-mimicking chemical) increases from very low to much higher doses, entirely different arrays of genes are activated and inhibited, leading to a unique set of responses at low and high doses (Coser et al., 2003).

It is obvious that the expectation is not that no effect will occur at high doses but that qualitatively different types of responses will occur at low and high doses. One basis for this prediction is that, at higher and higher doses, ligands for estrogen receptors, such as BPA, begin to bind to other receptors, for which they have a lower affinity. For example, at doses above those required to initiate many estrogenic responses, BPA binds to androgen receptors (Lee et al., 2003) and thyroid hormone receptors (Moriyama et al., 2002; Ishihara et al., 2003). With regard to the interaction between BPA and estrogen receptors, it is well known that, for a specific hormone-mediated response, the magnitude often decreases at doses that saturate receptors relative to doses below the K_d. The consequence is that both the receptor numbers and the magnitude of response can be "down-regulated" at high doses of hormonally active chemicals and drugs, while stimulation of response occurs at lower, physiologically relevant doses. In addition to high-dose inhibition of receptors, low, physiologically relevant doses of the same chemical or drug can stimulate an increase in receptors, and both of these phenomena contribute to inverted-U dose–response curves. The consequence is that low-dose hormonal effects of BPA and other chemicals cannot be assessed by conducting studies that examine only very high toxic doses, which is discussed in detail elsewhere (Welshons et al., 2003).

4. Evidence of bias in industry-funded research on BPA

In Table 2 we show the reported outcome of published studies (harm vs. no harm) in relation to source of funding (government vs. chemical corporations). Not one industry-funded in vivo study has led to the conclusion that

Table 2
Source of funding in relation to reported outcome of harm or no harm in low-dose animal studies with bisphnol A

Source of funding	Reported study outcome		
	Harm	No harm	Total
Government	109 (92%)	10 (8%)	119
Chemical corporations	0 (0%)	11 (100%)	11
Total	109	21	130

observable effects occur in response to low doses of BPA, while over 90% of the 109 government-funded in vivo studies conclude that such effects do, in fact, occur (including virtually all of the effects not found in industry-funded studies). Most of the studies reporting the absence of adverse effects from independent, government-funded scientists used the CD-SD rat as the test animal or looked for effects in the uterus that do not occur in response to BPA, which is discussed elsewhere (vom Saal and Hughes, 2005).

In more detail, of the 130 in vivo studies identifying the hazards associated with low-dose BPA exposure in experimental animals that have been published as of July 2005, there are 21 publications that report no significant effects of BPA, while the remaining 109 studies report adverse effects; in most cases there are multiple studies confirming positive results for outcomes not found to be affected by BPA in the studies reporting no harm (Table 1). An important aspect of the studies reporting adverse effects as opposed to no significant effects of low doses of BPA is that, of the 21 studies that report no low-dose effects of BPA, 11 were funded by chemical corporations. No industry-funded study has reported finding significant effects of low doses of BPA in any experimental animal, even when this conclusion is clearly invalid based on established standards of experimental design and analysis. Thus, none of the 109 published studies that report a significant effect of low doses of BPA was funded by the chemical industry.

4.1. The US-FDA and US-EPA continue to ignore the published low-dose literature on BPA

In response to our publication (Nagel et al., 1997), several industry-funded studies were rapidly conducted, and the conclusion from each of these studies was that our findings that low doses of BPA caused permanent changes in reproductive organs had not been confirmed. This had the expected effect of creating doubt in the regulatory community that our results were repeatable; an article about this corporate strategy of creating doubt was recently published (Michaels, 2005). For example, in 1999, an interview with a US-FDA official, Dr. George Pauli, was published in the Endocrine/Estrogen Letter [Vol. 5, No. 10 (106), 5/20/99] with the title "FDA Unimpressed By Low Dose Claims". The interview reports that

> George Pauli, director of the division of product policy at the US Food and Drug Administration (FDA) said the agency is following the low dose issue closely and has seen no reason to take any actions. Addressing the bisphenol A issue, he said that 'it is troubling that people who appear in good faith to replicate [the vom Saal study] haven't been able to replicate those findings. When you have larger studies intended to replicate a smaller study, and when you do not see the effects, it certainly casts doubt on relying on one study and ignoring the larger ones,' Pauli said that FDA cannot take actions based on vom Saal's research until it has been replicated. 'Until you can replicate something, you can't interpret its significance'.

With regard to the issue of replication, a member of the chemical industry BPA task force also referred to our initial BPA findings (vom Saal et al., 1997, 1998) in an interview. It was reported that "had that research been duplicated, it would have meant the 'margin of safety' for BPA would be much less than previously thought for consumer products, said John Waechter, a Dow Chemical Co. scientist and a member of SPI's BPA task force who presented the results" (Toloken, 1998). In contrast to the implication that the chemical industry or the US-FDA would respond to published replications of our findings by changing their position, in complete disregard of the mounting evidence of adverse low-dose effects of BPA, the plastics industry continued to state that "the weight of scientific evidence clearly supports the safety of BPA and provides strong reassurance that there is no basis for human health concerns from exposure to low doses of BPA" (Bisphenol-A-Global-Industry-Group, 2003). More recently the position of the Polycarbonate Business Unit Executive Director was published in a letter in the August 11, 2005 Wall Street Journal, which stated that

> Government agencies world-wide, including the Food and Drug Administration and the European Commission's Scientific Committee on Food, have comprehensively reviewed the evidence and, in every case, found no reason to be concerned about adverse effects on human health from the use of products made from BPA.

In addition, officials within the US-FDA responsible for determining the safety of chemicals that can enter foods via leaching from plastic appear to remain totally unconcerned about the findings from the government-funded research showing adverse effects of low doses of BPA listed in Table 1. For example, in an interview subsequent to publication of an article reporting that BPA disrupted meiosis in mouse oocytes (Hunt et al., 2003), a US-FDA official stated that, with regard to concern about BPA within the US-FDA, "we don't have any reason to believe there's any effect" (Pearson, 2003).

In science, independent confirmation of results is required prior to acceptance by the scientific community, and lack of confirmation raises doubt about whether conclusions are valid. However, there is also a high level of awareness within the scientific community that one always needs to be aware of the potential for conflict of interest when the issue being debated concerns a chemical, drug, or product that generates billions of dollars in profits for corporations. BPA is one of the highest-volume chemicals in production and generates billions of dollars in profits, and the data in Table 2 document that there is an extreme bias in outcome of experiments with low doses of BPA based on source of funding for the research. This is identical to the now well-documented bias shown by the lead and tobacco industries in studies that they funded (Needleman et al., 1975; Barnes and Bero, 1996; Markowitz and Rosner, 2000; Ong and Glantz, 2001; Tong et al., 2005), although there are also many other examples (Michaels and Morforton, 2005), including the ongoing controversy about the drug Vioxx. As shown in Table 2, similar to these prior examples, about which much has been written, government-funded studies by scientists independent of chemical corporations have overwhelmingly confirmed our initial prediction that BPA has biological activity in vivo at doses far below the dose range of 50–1200 mg/kg/day that had been examined (Morrissey et al., 1987) prior to our study (Nagel et al., 1997).

Given the very large number of studies showing adverse effects of low doses of BPA in experimental animals, 100% of which were conducted by scientists not associated with or funded by chemical corporations, it is important to examine in detail the studies that have led to the conclusion by chemical corporations and their surrogates, and by the US-FDA and US-EPA, that low doses of BPA cause no adverse effects. For additional recent examples of claims by industry trade organizations, chemical industry employees, or others funded by chemical corporations that there is no published evidence of harm due to exposure to low doses of BPA and our responses to those claims see Gray et al. (2004), Purchase (2004), APM (2005), vom Saal and Hughes (2005), vom Saal et al. (2005), and vom Saal (2005b).

5. The need for appropriate positive controls: review of the design and analysis of studies reporting no effects of low doses of BPA

To interpret whether there is a positive or negative effect of a test chemical, such as BPA, appropriate negative and positive controls also have to be examined. Five different possible outcomes in experiments are shown in Fig. 1, which includes experiments with and without a positive control. The different panels in Fig. 1 are discussed in relation to results of two studies that did not include a positive control (Ema et al., 2001; Tyl et al., 2002), two studies that included a positive control that did not differ from the negative control or test chemical (Ashby et al., 1999; Cagen et al., 1999), and, finally, one study that included a positive control estrogenic chemical of known potency that revealed that the test animal being used was inappropriate for examining effects of BPA due to very low sensitivity to exogenous estrogen (Yamasaki et al., 2002).

5.1. Experiments with no positive control

Fig. 1A shows an experiment in which only a negative control and test chemical are included, and there is no significant difference in response between the negative control and the test chemical. The NTP panel criticized investigators (for example Tyl et al., 2002) who reported that BPA caused no significant effects at low doses based on conducting studies without any positive control. In such experiments, all that can be legitimately stated is that the investigators failed to find any effects. Without appropriate positive control findings, interpreting the reason for purely negative results is not possible. It is important to also note that the experiment by Tyl et al. (2002) was criticized for using a model animal that other research has shown to be far less sensitive to any estrogen than other rat or mouse models, which is discussed in detail elsewhere (vom Saal and Hughes, 2005). The Tyl et al. (2002) study also used a type of animal feed that has been deemed inappropriate for use in studies of estrogenic chemicals due to very high variability in background estrogenic activity, which is also discussed in more detail elsewhere (Thigpen et al., 2003; vom Saal et al., 2004). Inclusion of an appropriate positive control by Tyl et al. would have allowed a determination of whether the failure to find effects of BPA was due to the lack of activity of BPA or to a lack of sensitivity of the animal model and/or estrogenic contamination by the feed that was used. The chemical industry and the senior author of this industry-funded study have drawn the strong positive conclusion that this study demonstrates that BPA is "safe" within the range of human exposure (Bisphenol-A-Global-Industry-Group, 2003; Tyl, 2003; Gray et al., 2004).

If one conducts a study with only a negative control and a test chemical and significant differences are found, it is legitimate to conclude that, relative to the negative control, the test chemical is significantly different. In industry-sponsored studies to assess the safety of chemicals already in commerce and generating billions of dollars per year in revenues (such as BPA), it cannot reasonably be argued that there is a very strong desire by these industries to have the chemical found to be unsafe, which would then result in a loss of profits from sales of the chemical. In studies in which a negative outcome is actually being predicted or at least hoped for by a particular industry that funds the study (Ashby et al., 1999; Cagen et al., 1999; Elswick et al., 2000; Tyl et al., 2002), it is essential to include a positive control group that shows positive effects on endpoints being measured and against which potentially negative findings for the test chemical can be compared and shown to be significantly different.

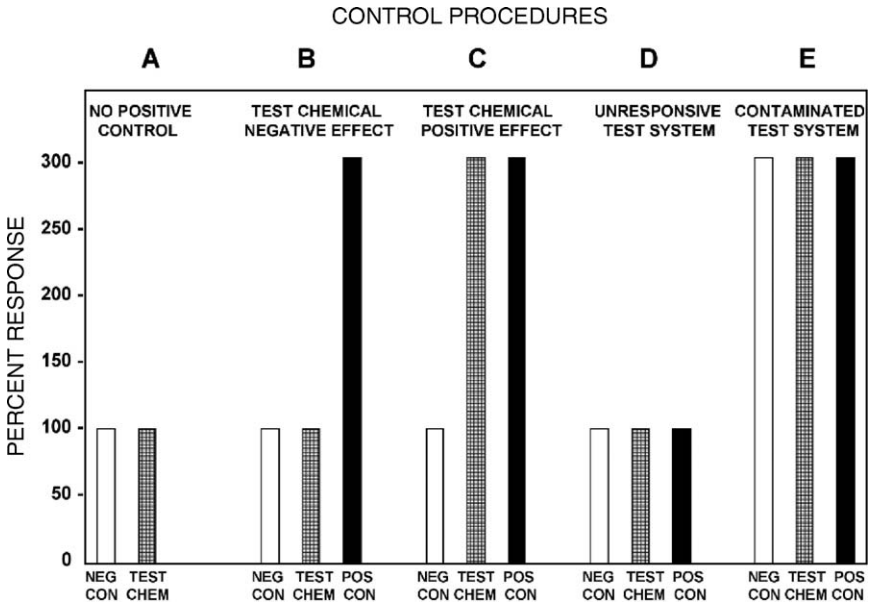

Fig. 1. Schematic diagram showing the response (such as prostate size) to a negative control (vehicle only), a test chemical (such as BPA), and a positive control chemical (such as DES). (A) An experiment in which only a negative control and a test chemical are examined, and no positive control is included. Negative results from this experiment cannot be interpreted, and statements that the test chemical is "safe" are inappropriate. (B) An experiment in which the test chemical did not differ from the negative control, while the negative control and positive control did differ. This is not typical of findings that have been reported for the effects of BPA and DES on the male reproductive system. (C) The system being examined is responsive to the dose of the positive control that was used, and a response of equal magnitude was observed at the dose of the test chemical that was used. This is representative of the data from experiments in the vom Saal and Gupta laboratories with BPA and DES (vom Saal et al., 1997, 1998; Gupta, 2000a) and from the Iguchi laboratory for effects on puberty (Honma et al., 2002) and the Aou laboratory for effects on brain structure and behavior (Kubo et al., 2003). (D) An experiment in which no response to the dose of the positive control or test chemical was observed. The conclusion is that within the parameters of the experiment, the system being investigated is unresponsive to estrogenic chemicals. (E) A system that could be assumed to be unresponsive to either the positive control or the test chemical. However, comparison of the negative controls in A, B, C, and D as opposed to E reveals that the entire experiment in E was "contaminated." The negative controls in E were not at historic values for negative controls but rather were at the historic value for animals maximally stimulated by estrogens (estradiol, ethinylestradiol, or DES) during fetal life. This represents the data from the experiments published by Ashby et al. (1999).

In sharp contrast to these basic principles of experimental design and analysis, it is a common event in toxicological studies conducted by the chemical industry for purposes of reporting about chemical safety to regulatory agencies to provide only negative results from a study in which no positive control was included but from which positive conclusions of safety of the test chemical are drawn (Tyl et al., 2002). In these studies, when the investigators find what those funding the research desire them to find, the fact that in science it is considered a violation of the scientific process to draw positive conclusions from uniformly negative data is simply ignored.

How does one choose a positive control in an experiment? For chemicals about which nothing is known about the mechanism of action, it is obviously not possible to determine an appropriate positive control for a specific response mechanism. However, it has been known since 1936 that BPA is a full estrogen agonist (Dodds and Lawson, 1936), and in experiments seeking to determine whether BPA at a specific dose causes estrogenic effects, there is no excuse for not including a well-characterized positive control estrogen appropriate for the mode of administration being used. The US-EPA was charged by the US Congress in 1996 (the Food Quality Protection Act and the Safe Drinking Water Act) with establishing a set of screening and testing procedures to determine whether chemicals had endocrine-disrupting activity. There now exist screening procedures for three classes of endocrine-disrupting chemicals: estrogen and antiestrogen, androgen and antiandrogen, and thyroid and antithyroid activity. Once an initial screen has established one of these types of endocrine-disrupting activity for a chemical, appropriate controls can be included in experiments that determine whether specific responses caused by a chemical are consistent with responses to the positive control.

For estrogenic effects of BPA based on oral administration, an appropriate positive control would be DES or ethinylestradiol, as demonstrated in a number of publications (Gupta, 2000a; Honma et al., 2002; Kubo et al., 2003; Nikaido et al., 2004; Timms et al., 2005). The use of estradiol in an experiment in which it is orally administered is not appropriate, since, unlike ethinylestradiol which was designed to be orally active, little estradiol (~3%) is absorbed through the gut. We have examined effects of estradiol by administering it in Silastic capsules and then measuring total and free levels of estradiol in maternal and fetal serum in comparison to effects on offspring. We then compared the effects of estradiol with results based on a

wide range of doses of DES administered orally (Montano et al., 1995; vom Saal et al., 1997). Using this technique we were able to establish the physiologically active dose range for DES in relation to total and free levels of serum estradiol in fetal mice (Welshons et al., 1999). We now use 0.1 μg/kg/day DES as our positive control in low-dose studies of the effects of estrogenic chemicals on fetal development in mice (Timms et al., 2005). Implanting a capsule containing estradiol as a positive control would be appropriate for the method used by Markey and colleagues where BPA is administered tonically by capsule (Markey et al., 2005).

5.2. Significant effect for the positive control but not the test chemical

In Fig. 1B a negative control, a test chemical, and a positive control are included. In this situation the test chemical does differ significantly from the positive control but is not significantly different from the negative control. With this finding it would be justified to conclude that the test chemical does not cause the estrogenic effect demonstrated to be caused by a positive control, such as DES. If this finding were, in fact, typical of results in experiments with BPA and DES or experiments with BPA and ethinylestradiol as the positive control, they would justify the conclusion that BPA did not produce the positive effect observed for the positive control. This finding would thus justify stating that BPA was not acting as an estrogenic chemical in the assay at the doses tested. However, the finding depicted in Fig. 1B is not typical of the published literature on BPA. None of the experiments funded by the chemical industry that included DES as a positive control produced findings similar to those in Fig. 1B, even though they are cited as showing that low doses of BPA are safe.

5.3. Similar efficacy of BPA and DES

In studies in which both BPA and DES were examined, findings similar to those in Fig. 1C have been reported. The potency of BPA has typically been 100- to1000-fold lower than DES based on the doses used in some of these studies (Gupta, 2000a; Honma et al., 2002; Suzuki et al., 2002; Kubo et al., 2003; Evans et al., 2004; Nikaido et al., 2004; Timms et al., 2005). It is interesting that, similar to BPA, DES shows limited binding to plasma estrogen-binding proteins in both rodents and humans (Sheehan and Young, 1979; Sheehan and Branham, 1987). In some cell culture assays in which plasma-binding proteins are not an issue, BPA has actually been reported to result in a greater response relative to DES or estradiol at some doses (Wozniak et al., 2005).

5.4. Unresponsive test system

Fig. 1D shows a situation in which the negative control, test chemical, and positive control show no differences. Fig. 1D is consistent with the findings reported by Yamasaki et al. (2002) that compared responses to ethinylestradiol and BPA in the CD-SD rat. The appropriate interpretation is that the response being measured is not affected by estrogenic chemicals within the dose range administered, at the time point examined, in the strain of animal examined, or for a variety of other reasons. The NTP panel stated that with regard to "a study in which the positive control does not produce the expected positive response. The prudent course of action in such cases may be to declare the study inadequate and repeat it, regardless of the experimental outcome in the test groups" (NTP, 2001, pp. 5–14). We have reviewed elsewhere the insensitivity of the CD-SD rat to even the potent estrogenic drug ethinylestradiol and the insensitivity that this predicted in response to low doses of BPA (vom Saal and Hughes, 2005).

5.5. Test system contaminated with estrogen

The results of the Ashby et al. (1999) and Cagen et al. (1999) studies are similar to those depicted in Fig. 1E, which suggests that the test system was maximally contaminated with estrogen from some source. This prediction is based on the negative control animals showing responses that do not differ from the response shown to DES at a dose of 0.2 μg/kg/day. Thus, while both Ashby et al. (1999) and Cagen et al. (1999) included a positive control (DES) in their studies, when the positive control was not different from the negative control, they ignored the consequence of this negative finding. In both of these studies, the authors concluded that BPA had no effects and reported to the public (and regulatory agencies) that published reports that exposure to BPA during fetal life altered the reproductive system in mice could not be repeated. As noted above, Dr. George Pauli at the US-FDA appeared to be unaware that the results of these studies were being misrepresented to regulatory agencies and the public, and he used these finding to reject the possibility that there were reliable low-dose effects of BPA. There is no evidence that Dr. Pauli or any other official at the US-FDA or US-EPA has acknowledged that there is now extensive independent evidence for low-dose effects of BPA and that studies funded by chemical corporations need to be carefully examined for errors in design and analysis.

The NTP Low-Dose Peer Review Panel addressed the issue of the inclusion of a positive control in toxicological studies, and it was noted that an appropriate positive control in studies of environmental chemicals with estrogenic activity would be the well-characterized drug DES within the dose range of 0.2–2 μg/kg/day, which we and others have used in studies. The panel noted that "For those studies that included DES exposure groups, those that showed an effect with BPA showed a similar low-dose effect with DES (e.g., prostate and uterus enlargement in

mice), while those that showed no effect with BPA also found no effect with DES'' (NTP, 2001, p. iii).

Fig. 1E shows an outcome markedly different from that in Fig. 1D in that in Fig. 1E, all animals show maximum responses (for example, maximum prostate size). An important aspect of this possible outcome is that there is a maximum amount that the prostate can be increased in size by developmental exposure to estrogen, after which it begins to decrease in size as the dose increases, forming an inverted-U dose–response curve (vom Saal et al., 1997; Gupta, 2000a; Putz et al., 2001; Timms et al., 2005). An appropriate interpretation of the results in Fig. 1E is that all animals in the experiment, regardless of treatment condition, were exposed to an estrogenic contaminant that maximally stimulated estrogenic responses. For example, if the negative control animals show maximally enlarged prostates, then detecting an effect of BPA or DES on stimulation of the prostate is not possible. A detailed discussion of this issue, with examples from experiments, is provided in a companion paper (Welshons et al., 2003). The source of the contamination needs to be discovered and eliminated to conduct studies of the effects of BPA or other estrogenic chemicals.

5.6. *Misrepresentation of the positive control DES as another test chemical due to failure to find an effect of DES*

A difficult situation arises for those reading an article when investigators actually design an experiment that produces results like those shown in Fig. 1E (when the positive control group fails to show an effect that is different from the negative control group) and the investigators do not acknowledge that the positive control in the study was actually a ''positive control.'' Instead, the ''positive control'' chemical (DES) is presented as if it had been included in the experiment as just another test chemical. Such an experiment would then look like the experiment shown in Fig. 1A (but with multiple test chemicals) rather than the experiment shown in Fig. 1E. As noted by the NTP panel, knowing that the positive control failed changes the conclusions that are drawn from the results.

Two industry-designed and -funded studies of BPA in mice (Ashby et al., 1999; Cagen et al., 1999) will be discussed here as examples of how research findings can be misrepresented by not identifying that the experiment included a positive control condition at the time that the experiment was designed. What is important about these two studies by Ashby et al. and by Cagen et al. is that they both used the same design. The design of the studies conducted by both Ashby et al. and Cagen et al. was presented to the public at a press conference held by the SPI in Washington, DC in October 1998. In this public presentation including handouts containing specifics of the design of the ''SPI/CEFIC Protocol Overview,'' the SPI identified that they had included a positive control group in designing their experiment, which they claimed matched the study reported by vom Saal et al. (1998). Specifically, it was reported that in the industry studies there was a ''Positive Control Group—DES at 0.2 μg/kg/day.'' In addition, Cagen et al. (1999) stated in their article that ''Since the present study was specifically undertaken to repeat the experiments of Nagel et al. (1997) and vom Saal et al. (1998), the study was designed to duplicate the procedures detailed in those reports as closely as possible.'' What is interesting about this statement is that the Cagen et al. study was started with the intention of examining the reproductive organs in males when they reached 6 months of age, similar to the studies in the vom Saal laboratory and the study by Ashby et al. However, the initial set of mice to be used in the Cagen et al. study (that were exposed during prenatal life to BPA and DES) were killed and presumably discarded without any results being reported, and another set of animals was then only reared to 3 months of age, at which time they were examined by Cagen et al. The basis for this action was not explained, but it is obvious that a direct comparison of prostate size of animals in the Cagen et al. (1999) study and the Nagel et al. (1997) study is not possible due to the difference in the age of the animals. However, the size of the prostate for all 3-month-old animals (regardless of treatment condition) used in the Cagen et al. study is equal to that for 6-month-old males and much larger than 50-day-old males from the same strain (Thayer et al., 2001).

The studies by Cagen et al. and Ashby et al. involved administration of BPA to pregnant CF-1 mice and examination of the reproductive organs in male offspring during postnatal life. Of importance is that in these two studies no effect of either the BPA or the positive control estrogenic chemical DES on the prostate was found relative to the negative controls (Ashby et al., 1999; Cagen et al., 1999). However, the body weights of the negative control animals (and BPA- and DES-exposed animals) in the Ashby et al. (1999) study were markedly greater than those of the negative control animals in the studies in which there were effects of BPA, and effects of the positive control chemical DES (Nagel et al., 1997; vom Saal et al., 1997). In addition, in the Ashby et al. study, the prostate in negative control animals was significantly enlarged relative to prostate weights reported for the negative control animals in studies conducted in any experiment in the vom Saal laboratory (Nagel et al., 1997; vom Saal et al., 1997; Welshons and vom Saal, 1998).

In neither of the journal articles published by Ashby et al. (1999) and by Cagen et al. (1999) was it clearly stated that that the animals exposed to DES at a dose of 0.2 μg/kg/day had been included in the design of the study as a positive control. When the positive control does not show a positive effect, one has to decide whether the system being studied is completely unresponsive to estrogenic stimulation (Fig. 1D) or whether there was contamination by estrogen that interfered with detection of an estrogenic response (Fig. 1E). The purpose of including negative and positive controls for estrogenic activity and making

comparisons to historic data on negative and positive control values from prior experiments is to be able to make this determination (Welshons et al., 2003).

The findings by Ashby et al. (1999) are shown in Fig. 2 in relation to data from animals tested at the University of Missouri in the vom Saal laboratory (Nagel et al., 1997). The comparison is justified by the fact that the technician in Ashby's lab was trained to remove the prostate by a student in vom Saal's laboratory, since there was no expertise in the Zeneca laboratory to conduct this study on developmental effects of estrogen on the male reproductive system in mice (this training was acknowledged in the published article). This side-by-side comparison should have led Ashby et al. to conclude that all animals in the study (negative controls, BPA-exposed, and DES-exposed) had been maximally exposed to estrogenic chemicals from some unknown source. In fact, the NTP Low-Dose Peer Review panel stated that "Careful examination of the raw data indicates that certain parameters in the control animals were different in studies that observed and did not observe low-dose effects of BPA. In particular, the control body weights and prostate weights differ between some studies, e.g., some of the Ashby studies and the vom Saal studies. This raises the theoretical possibility that tissues may have already been maximally stimulated by estrogens…" (NTP, 2001, pp. 1–8). In contrast to the conclusion of the NTP Low-Dose Peer Review panel, Ashby et al. claimed in their publication that "although the weights of the prostate glands in the present experiment were marginally higher than those described by vom Saal and colleagues, they were not substantially different" (Ashby et al., 1999). As shown in Fig. 2, this claim is not accurate and was disputed by the NTP Low-Dose Peer Review panel. Cagen et al. simply ignored the possibility that the findings could be due to the presence of a contaminant.

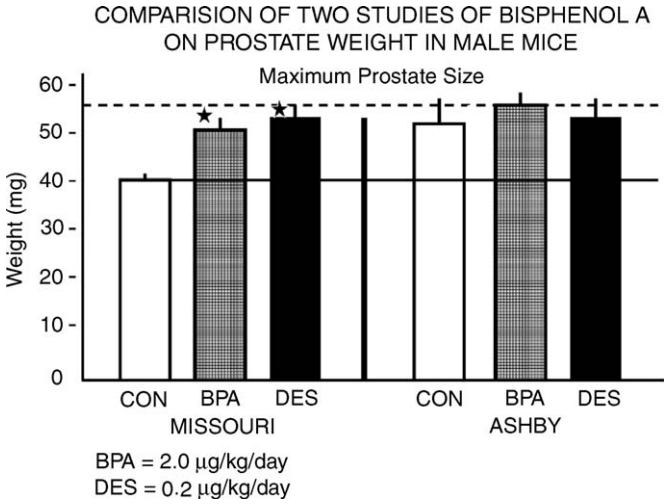

Fig. 2. Findings from two separate experiments conducted in the vom Saal laboratory at the University of Missouri (Nagel et al., 1997; vom Saal et al., 1997) and from a study designed by the plastic industry (Ashby et al., 1999). The conclusion by both Ashby et al. and the chemical industry about the Ashby et al. study is that BPA caused no effect on the prostate. However, side-by-side examination of the findings from the initial University of Missouri studies and the Ashby et al. study by the NTP Low-Dose Peer Review panel led to the conclusion that all animals in the Ashby et al. study appeared to have been exposed to an estrogenic contaminant, with the consequence that males in all groups, negative controls, BPA-exposed, and DES-exposed, had significantly enlarged prostates relative to negative control values from the same mouse stock examined at the same age at the University of Missouri (NTP, 2001, pp. 1–8). *Indicates statistically different from negative controls in the Nagel et al. study and vom Saal et al study conducted at the University of Missouri.

Importantly, the Ashby et al. and Cagen et al. experiments have been presented to regulatory agencies by the chemical industry as exact replicates of the vom Saal laboratory experiments. In sharp contrast to this false representation, the NTP Low-Dose Peer Review panel raised the possibility that, since both Ashby et al. and Cagen et al. used types of feed different from the commercial feed that had been used in the vom Saal laboratory, the feed could be the source of contamination in the Ashby et al. and Cagen et al. experiments, resulting in an increase in prostate size in all animals. This prediction was quite likely correct, at least for the Cagen et al. study (Thigpen et al., 2003; vom Saal and Hughes, 2005).

5.7. *Variability in negative controls from one experiment to another*

An interesting argument is that variability in control values from one experiment to the next invalidates any positive findings on low-dose effects of BPA (Ashby et al., 2004). This assertion violates basic assumptions about the design and analysis of research if appropriate controls are included and animals are randomly assigned to control and experimental groups. There are many factors that are well known to influence the magnitude of a response. For example, time of day can influence responses impacted by plasma corticosterone (Montano et al., 1991), and type of feed (feed with and without isoflavones) can influence estrogen-mediated responses (Thigpen et al., 2003; Wang et al., 2005). The issue of variability in estrogenic activity in batches of the same feed has also recently been recognized as a critical issue affecting research on estrogenic endocrine disruptors and other types of research (Thigpen et al., 2003; vom Saal et al., 2004). Genetic differences are a major factor in differences in responses to chemicals (Ohsako and Tohyama, 2005). Since there are so many factors that can alter the magnitude of various responses, it is not surprising that variation in the magnitude of a particular response will occur from experiment to experiment, particularly if different strains of animals are used (Ohsako and Tohyama, 2005). Obviously, researchers seek to control this variability to the greatest extent possible. However, for toxicological studies, background variability in negative control values does not invalidate the study as long as the experimental system remains sensitive to the positive control for the agent being tested. The positive

control is the key to being able to identify whether the system has become more or less sensitive to a specific agent as the negative control mean changes from one experiment to another.

The degree to which variability in negative control values creates a significant problem can depend on the shape of the dose–response curve. We will present two examples of situations in which a shift in the mean for the negative control group can eliminate the ability of the experiment to detect an effect. For the first example, the sensitivity of the prostate in a particular experiment to estrogenic stimulation (by BPA or any other estrogen) is reflected by the size of the prostate in negative controls, since, as background estrogenic activity increases, the size of the prostate in the negative controls will increase, thus reducing the magnitude of the effect that can be achieved by adding more estrogen (either DES or BPA) to the system. The problem is that the relationship between fetal exposure to estrogenic chemicals and prostate development forms an inverted U, as has been shown in numerous experiments in which exposure during development to low doses of estrogenic chemicals has a stimulating effect, while at much higher doses the stimulating effect does not occur and eventually a dramatic inhibition of prostate development occurs (vom Saal et al., 1997; Gupta, 2000a; Timms et al., 2005). As a result of this dose–response relationship, an upward shift in the mean for the negative control group can eliminate the ability of the experiment to detect a low-dose stimulatory effect, while high-dose inhibitory effects might still be observed. The sensitivity of the prostate in a particular experiment to estrogenic stimulation (by BPA or any other estrogen) is thus reflected by the size of the prostate in negative controls.

Ashby et al. (2004) proposed that a downward shift in the negative control mean would lead to a false positive effect of a chemical and thus that the magnitude of the negative control mean is related to the probability of a false positive finding. This proposition is illogical. The rule in toxicology is that the most sensitive test system should be used in assessing the hazards posed by a chemical, not the least sensitive test system. For an experiment examining the effect of BPA on development of the prostate, the downward shift in the negative control (for example based on the type of feed used) could result in a greater magnitude of the stimulatory effects of a chemical such as BPA or DES. It is very odd that a change in the sensitivity of the model system due to extrinsic factors could be confused by Ashby et al. (2004) as a basis for a false positive outcome.

Ashby et al. (2004) have also stated that, because values for prostate weight were different in a paper published by Nonneman et al. (1992) relative to other studies that were actually conducted by students working in the vom Saal laboratory (Nagel et al., 1997; vom Saal et al., 1997), the increase in prostate size caused by estrogenic chemicals would represent a false positive outcome. However, in contradiction to the claim by Ashby et al. (2004), Nonneman was not a graduate student in the vom Saal laboratory and, based on the weights of the prostates in Nonneman et al. (1992), obviously did not use the same techniques to dissect the prostate as those used in subsequent experiments conducted in the vom Saal laboratory. However, this is irrelevant, since in all cases dissections were conducted blind by only one investigator, and any possibility of experimenter bias was thus eliminated within each independent experiment. Thus, within each experiment, the results are valid and have now been confirmed by independent studies.

A similar criticism was made by Ashby et al. (2004) concerning findings from studies conducted in the Tohyama laboratory at the Japanese NIH, in which the mean daily sperm production from different strains of rats used in different experiments conducted at different times was not similar. Ashby et al. (2004) stated that, because of this variability in control values in different experiments with different strains of rats, findings concerning the effect of BPA reported on one of these strains represented a false positive finding. This again is illogical, and this issue is discussed in a published response by Tohyama and colleagues (Ohsako and Tohyama, 2005).

What is clear from the data presented in Fig. 3 is that, in attempting to replicate the BPA finding from the Tohyama laboratory (Sakaue et al., 2001) with the same strain used in the study conducted in the Tohyama laboratory and the same type of feed, the mean daily sperm production value for the negative controls in the Ashby et al. (2003) study was identical to the maximum inhibitory effect reported by the Tohyama laboratory after exposure to BPA. However, in the abstract, Ashby et al. (2003) stated that

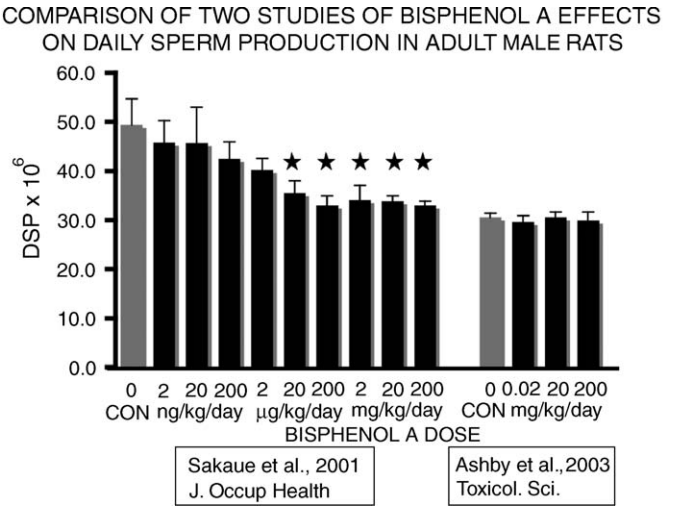

Fig. 3. Side-by-side comparison of an initial study conducted by Sakaue et al. (2001) and an attempt by Ashby et al. (2003) to replicate the study using selected doses from the initial study by Sakaue et al. Note that the only significant difference in the results of the two studies is in the daily sperm production (DSP) for the control (CON) group, suggesting that daily sperm production was maximally suppressed by a contaminating estrogen in the Ashby et al. study. *Indicates significantly different from controls in the Sakaue et al. study.

"No explanation for our failure to replicate the effects reported by Sakaue et al. is evident." If a positive control, such as DES, had been included in the Ashby et al. (2003) experiment, it would have allowed this group to determine whether daily sperm production in the negative controls in the Ashby laboratory was maximally suppressed by some contaminating factor and the animals were thus insensitive to the suppressive effect of any estrogen. Instead, by not including a positive control, Ashby et al. (2003) concluded that low doses of BPA caused no significant effects, consistent with every other published report from the Ashby laboratory about BPA and in marked contrast to numerous other published studies reporting in vivo effects of similar low doses of BPA on testicular sperm production in mice and rats (vom Saal et al., 1998; Talsness et al., 2000; Al-Hiyasat et al., 2002; Chitra et al., 2003; Aikawa et al., 2004; Takagi et al., 2004; Toyama and Yuasa, 2004) and on the testes in a wide variety of aquatic animals (Table 1; vom Saal, 2005a).

The shape of the dose–response curves for the effect of BPA and other estrogenic chemicals on the prostate and daily sperm production are presented in Fig. 4. Given the above facts and the elementary design and statistical issues being discussed, it is astonishing that the Ashby et al. (2003) study and the prior Ashby et al. (1999) study described above are still widely interpreted within the toxicological community as providing strong evidence that fetal exposure to low doses of BPA does not influence testicular sperm production or prostate size or have any other effects in either males or females. For the females examined in the Ashby et al. (1999) study, uterine weight was examined without any attempt to control for stage of estrous cycle by ovariectomizing and implanting the females with estradiol or by using prepubertal females, which would have revealed permanent effects of developmental exposure to estrogenic chemicals on the uterus (Alworth et al., 2002; Newbold et al., 2004). The uterine weights for the adult, gonadally intact, cycling negative control female mice in the Ashby et al. study ranged from 67 to 290 mg, and a power analysis revealed that the number of animals examined did not provide sufficient power to find the predicted magnitude of effect for either DES or BPA. It is thus not surprising that the conclusion by Ashby et al. was thus that prenatal exposure to either DES or BPA had no permanent effects on the uterus. The profound flaw in the design of this study should have precluded this experiment from being published.

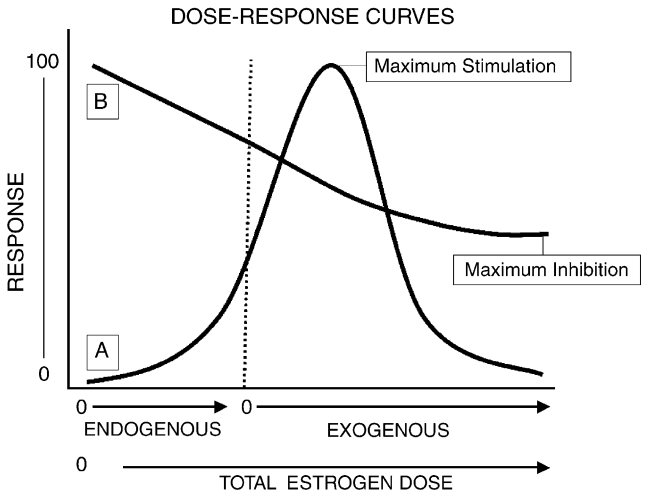

Fig. 4. Schematic diagram of two different dose–response curves for the effects of estrogenic chemicals in males. (Curve A) Relationship of dose during fetal life (via the dam) to prostate size in male offspring in adulthood. (Curve B) Relationship of dose in adulthood to daily sperm production. In both cases, if the total amount of estrogenic activity (total dose of endogenous estradiol and exogenous estrogenic chemical) is high enough so that the response of the negative controls is already at the maximum response point (maximum stimulation for curve A or maximum inhibition for curve B) then no stimulating effect on the prostate or inhibitory effect on DSP could be observed due to administration of an estrogenic chemical such as BPA.

An increase in variance is thus important as this impacts power and requires a larger sample size to achieve the same confidence level for rejecting the null hypothesis. An obvious concern with studies conducted by large commercial toxicological testing corporations (Tyl et al., 2002) is the number of technicians assigned to measure a particular outcome (R. Tyl has acknowledged the use of multiple dissectors to remove organs). When more than one technician makes a measurement, it is essential to provide the amount of variability in the measurement contributed by different technicians, which may be large enough to produce false negative findings.

5.8. *Positive effects of BPA on the prostate reported as negative*

In October 2000 a panel of 36 experts was selected by the NTP at the request of the US-EPA to review articles relating to the low-dose issue in toxicology that were published or accepted for publication. The NTP panel reviewed a study conducted at the Chemical Industry Institute of Toxicology (CIIT), now called the Centers for Health Research, concerning the effect of developmental exposure (during gestation and lactation) to BPA on the prostate and other reproductive organs in male rats (Elswick et al., 2000). Importantly, this review was conducted prior to publication of the article. This study by Elswick et al. that was funded by the chemical industry was prompted by our prior observation in mice that fetal exposure to very low doses of BPA altered development of the prostate, seminal vesicles, epididymis, and testicular sperm production (Nagel et al., 1997; vom Saal et al., 1998). Elswick et al. administered pregnant and lactating Sprague–Dawley CD rats BPA in their drinking water (0, 0.005, 0.05, 0.5, 5.0, or 50 mg/L) from gestation day 2 to postnatal day 21. The authors estimated that the rats in these treatment groups consumed BPA at average doses of 0, 0.001, 0.01, 0.1, 1.0, and 10 mg/kg/day, respectively. The authors concluded that there were no effects of BPA on the weight of the ventral prostate or on any other measure in

adult male offspring. The title, "Effect of different sampling designs on outcome of endocrine disruptor studies" provided no clue as to objectives of the study or the actual findings. The journal article title also was markedly different from the abstract title when the findings were first presented at a meeting "Effects of perinatal exposure to low doses of BPA in male offspring of Sprague–Dawley rats." (Toxicol. Sci. 54 (Suppl.): 256A).

The NTP Low-Dose Peer Review Statistics subpanel stated that the authors' reasons for this conclusion of no effect of BPA were "flawed," "illogical," and "misleading" (NTP, 2001, pp. A-89–A-91). Specifically, the NTP panel stated in their report, posted on the internet (NTP, 2001), that with regard to the prostate findings "The 0.05 mg/liter (0.01 mg/kg/day; $P<0.01$), the 5 mg/liter (1 mg/kg/day; $P<0.0001$) and the 50 mg/liter (10 mg/kg/day; $P<0.02$) groups were significantly increased in ventral prostate size relative to controls" (p. A-86). The findings presented in Fig. 5 are based on the analysis conducted by the NTP panel, and this figure and these conclusions did not appear in the paper published by Elswick et al. These statements by the NTP Low-Dose Peer Review panel were made to the authors prior to publication of the article but were ignored.

To justify discounting their positive findings, the authors of this CIIT study proposed that, by sampling one male per litter, they had produced a false positive finding. With regard to this attempt to discount significant findings, the NTP panel stated that "To suggest that using fewer pups per litter (thereby increasing the variability) would lead to increased findings of statistical significance is illogical" (NTP, 2001, p. A-90). The published article also contained a computer simulation that the authors suggested justified the conclusion of no effect of BPA. But, in reviewing this simulation, statisticians on the NTP Low-Dose Review Peer panel concluded that "The Statistics Sub-panel believes that the simulation study is seriously flawed and gives a misleading impression of the statistical benefits associated with a multiple-pup per litter experimental design" (p. A-90). It would be unfortunate if other researchers designed studies based on the bizarre suggestions by Elswick et al.

De spite this blistering review by the NTP Low-Dose Peer Review panel, the American Chemistry Council (ACC) appended a letter to the report when it was submitted by the NTP to the US-EPA (the report contained a public comment section), since the US-EPA had commissioned the review by the NTP. The ACC letter states that "A very large study conducted by Welsch (Elswick and Welsch (and Janszen), 2000) using multiple pups per litter also found no BPA effects on prostate weight at 0.005, 0.05, 0.5 or 5 mg/liter drinking water (0.001 to 10 mg/kg/day)." (Appendix C of the NTP report on Low Dose Effects, Public Comments, p. C-89). This statement directly contradicts the conclusions drawn by the review panel in the report to which this letter was attached. The authors of this article thus misrepresented their findings in the published article, and the ACC misrepresented the actual findings in their letter to the US-EPA.

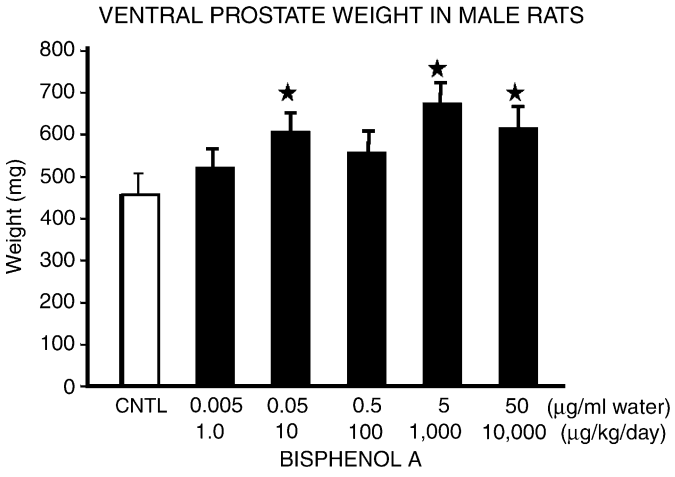

Fig. 5. Results from a study by Elswick et al. (2000) in which BPA was administered via the drinking water to pregnant and lactating rats. There is a significant stimulating effect of low doses of BPA on the ventral prostate in male offspring, replicating findings in mice of a stimulating effect of BPA on the prostate due to fetal exposure to doses between 2 and 50 µg/kg/day (Nagel et al., 1997; vom Saal et al., 1998; Gupta 2000a). The data shown are not presented in the publication by Elswick et al. but instead represent a reanalysis of the data based on a review of this paper by the National Toxicology Program Low-Dose Peer Review panel. While the authors concluded that these findings were not significant, the NTP panel was highly critical of the authors' conclusions and instead, determined that the animals treated with the doses indicated by * were statistically different from the negative controls (NTP, 2001, p. A-89).

5.9. *False positives and false negatives*

Modern scientific research is based is based on the principle of "falsifiability," which was proposed at the beginning of the 20th century by Karl Popper. In experimental research scientists test whether the hypothesis that the observed results come from the same distribution (the null hypothesis) can be rejected with a specific level of confidence. This is accomplished by "statistical significance testing." The hypothesis that results all come from the same distribution (the same population) can be disproved or falsified only at some specified level of confidence, it can never be proven to be correct. That is, you can find with some level of confidence that the distributions being sampled from different treatments appear not to be the same, but you can never prove that they do not differ. You thus cannot draw positive conclusions of proof from negative findings of no statistically significant difference.

In statistical significance testing there are two potential types of error: Type I error occurs if the null hypothesis is true and is rejected (false positive) and a Type II error occurs if the null hypothesis is false but is not rejected (false negative) (Rosenthal and Rosnow, 1991, p. 35). In toxicology, regulatory agencies should be highly concerned with false negatives (Type II errors), while the industries producing products are generally most concerned with false

positives (Type I errors), at least until the litigation begins (Markowitz and Rosner, 2000; Ong and Glantz, 2001; Michaels, 2005).

Scientists are primarily concerned with making Type I (false positive) errors, as careers are lost reporting a finding that cannot be replicated by other scientists with demonstrated competency to conduct the study. People associated with the scientific community who make a career of publicizing the fact that they cannot replicate findings published by dozens of independent laboratories would thus not be expected to exist, and, in fact, outside of the corporate scientific community (Tyl et al., 2002; Ashby et al., 2003) they typically do not. The inability of someone to replicate published findings that have been independently replicated by those with recognized expertise risks being viewed as their having a lack of technical competence, and for this reason most scientists are correctly very conservative about making the claim that a finding of no significant effects from his or her laboratory means that no other findings of significant effects should be accepted (Tyl, 2003).

We recommend that laboratories demonstrate effects with positive controls before initiating new studies. However, given that there can be variability from experiment to experiment in contaminants that can result in a loss of sensitivity of a model system, even one that had been working reliably for years (Wang et al., 2005), it is essential to conduct simultaneous positive controls in an experiment in which one would publish findings of absolutely no significant estrogenic effects for a test chemical such as BPA.

The issue of reliability (repeatability) of research findings is solved by independent replication where others with demonstrated competence doing similar experiments find the same thing. For example, exposure to BPA, estradiol, DES, and ethinylestradiol during development has been shown in multiple published studies to increase prostate size (Nagel et al., 1997; vom Saal et al., 1997, 1998; Elswick et al., 2000; Gupta, 2000a, b; Thayer et al., 2001; Timms et al., 2005). The recent report that exposure of Sprague–Dawley rats to 10 μg/kg/day BPA during the first 5 days after birth resulted in prostatic interepithelial neoplasia, which is considered an early phase of prostate cancer, in adulthood adds to this literature (Tang et al., 2005). The report of an absence of a significant effect of BPA, DES, or ethinylestradiol (at similar doses) on prostate development in rats or mice by other researchers, particularly those with no prior history in the field (Ashby et al., 1999; Cagen et al., 1999), then becomes suspect with regard to what relevant variables might have differed relative to the studies that found a significant effect.

6. Conclusions

Taken together, there is now a large "low-dose" literature that demonstrates that in many tissues in many species, BPA is a chemical with a much higher estrogenic potency than has been acknowledged by chemical corporations and regulatory agencies, since BPA elicits a wide range of effects at doses many orders of magnitude below doses previously predicted to cause no effect (IRIS, 1988). While many of the effects of BPA are mediated by the nuclear receptors for endogenous estrogen that act as ligand-activated transcription factors, there are now many studies showing that effects, such as extracellular calcium influx leading to activation of enzymes in target cells, also occur via, as yet, unidentified receptors that appear to be associated with the cell membrane (Quesada et al., 2002; Walsh et al., 2005; Wozniak et al., 2005; Zsarnovsky et al., 2005). There are also intriguing studies reporting unique effects of low doses of BPA that are not predicted by effects of other estrogenic chemicals, suggesting that, in addition to acting as a relatively potent environmental estrogen, BPA can cause some unique types of toxic effects through, as yet, unknown molecular mechanisms. For example, in the hippocampus, BPA has the paradoxical effect of acting to block the beneficial effects of estradiol on neuronal synapse formation (MacLusky et al., 2005). There are other reports indicating the potential to disrupt thyroid hormone action (Moriyama et al., 2002; Zoeller and Rovet, 2004) and that BPA acts as an androgen antagonist in the presence of the wild-type androgen receptor (Paris et al., 2002; Lee et al., 2003). However, the interaction with these other receptors appears to require higher doses than interactions with nuclear or nonnuclear estrogen receptors. An interesting finding is that very low part per trillion doses of BPA also cause proliferation of human prostate cancer cells via binding to a mutant form of the androgen receptor expressed in a subpopulation of prostate cancer cells, although higher doses in the part per billion range are without effect (Wetherill et al., 2002).

The misrepresentation of the findings by Elswick et al. (2000) by the ACC and the misrepresentation of the findings from the Ashby et al. (1999) and Cagen et al. (1999) studies (all proposing to have only nonsignificant findings with low doses of BPA) have been part of a successful campaign by the chemical industry to have the US-EPA and US-FDA ignore the conclusion of the NTP Low-Dose Peer Review panel that there was credible evidence for effects of some chemicals in the low-dose range. Specifically, in their report to the US-EPA, the NTP panel stated in 2001 that

> The findings of the NTP Panel indicate that the current testing paradigm used for assessments of reproductive and developmental toxicity should be revisited to see if changes are needed regarding dose selection, animal model selection, age when animals are evaluated, and the endpoints being measured following exposure to endocrine active agents (NTP, 2001, p. vii).

The EPA ignored this recommendation and concluded that more research was needed and that there would be no regulatory action with regard to BPA "until there is a better understanding of mechanisms" (EPA, 2002).

We suggest that a reevaluation that includes the studies listed in Table 1 and others being published every month not included in this review needs to be conducted by regulatory agencies. This should result in a reexamination by regulatory agencies of the use of BPA in products used for food and beverages and new policies to regulate the disposal of BPA-containing products in landfill. This latter recommendation is based on the results of studies conducted in Japan (Kawagoshi et al., 2003) and in the USA (Coors et al., 2003) showing that BPA accounts for the majority of estrogenic activity that leaches from landfills into the surrounding ecosystem. Our conclusion is based on the fact that mean blood levels of BPA in human fetuses are greater than levels of BPA in mouse fetuses due to maternal administration of doses of BPA that cause adverse effects in mice (Schönfelder et al., 2002a; Zalko et al., 2003).

While there are, as yet, relatively little published data on human health effects related to exposure to BPA, there are two epidemiological studies showing a relationship between blood levels of BPA and ovarian disease (Takeuchi et al., 2004) and recurrent miscarriage (Sugiura-Ogasawara et al., 2005). The existence of these findings is constantly denied by those presenting the views of the chemical industry. A recent example is the following:

> Typical human exposures to BPA are 100 times to 1000 times lower than the levels permitted by government guidelines—rules that are set way below actual safety levels. Human exposure levels are typically more than one million times lower than levels shown to be safe in experiments involving multiple generations of laboratory animals (Milloy, 2005).

The weight of evidence based on examination of the published studies concerning low-dose effects of BPA in experimental animals demonstrates the need for a re-evaluation of the prior estimate of the acceptable level of daily human exposure to BPA, which is currently 50 μg/kg/day in the United States.

Acknowledgments

Support was provided during the preparation of this manuscript by grants to F.vS. from NIEHS (ES11283) and to W.V.W. from NCI (CA50354) and University of Missouri (VMFC0018).

References

Adriani, W., Della Seta, D., Dessi-Fulgheri, F., Farabollini, F., Laviola, G., 2003. Altered profiles of spontaneous novelty seeking, impulsive behavior, and response to D-amphetamine in rats perinatally exposed to bisphenol A. Environ. Health Perspect. 111, 395–401.

Aikawa, H., Koyama, S., Matsuda, M., Nakahashi, K., Akazome, Y., Mori, T., 2004. Relief effect of vitamin A on the decreased motility of sperm and the increased incidence of malformed sperm in mice exposed neonatally to bisphenol A. Cell Tissue Res. 315 (1), 119–124.

Akingbemi, B.T., Sottas, C.M., Koulova, A.I., Klinefelter, G.R., Hardy, M.P., 2004. Inhibition of testicular steroidogenesis by the xenoestrogen bisphenol A is associated with reduced pituitary luteinizing hormone secretion and decreased steroidogenic enzyme gene expression in rat Leydig cells. Endocrinology 145 (2), 592–603.

Al-Hiyasat, A.S., Darmani, H., Elbetieha, A.M., 2002. Effects of bisphenol A on adult male mouse fertility. Eur. J. Oral Sci. 110, 163–167.

Al-Hiyasat, A.S., Darmani, H., Elbetieha, A.M., 2004. Leached components from dental composites and their effects on fertility of female mice. Eur. J. Oral Sci. 112, 267–272.

Aloisi, A.M., Della Seta, D., Ceccarelli, I., Farabollini, F., 2001. Bisphenol-A differently affects estrogen receptors-alpha in estrous-cycling and lactating female rats. Neurosci. Lett. 310 (1), 49–52.

Aloisi, A.M., Della Seta, D., Rendo, C., Ceccarelli, I., Scaramuzzino, A., Farabollini, F., 2002. Exposure to the estrogenic pollutant bisphenol A affects pain behavior induced by subcutaneous formalin injection in male and female rats. Brain Res. 937 (1–2), 1–7.

Alworth, L.C., Howdeshell, K.L., Ruhlen, R.L., Day, J.K., Huang, H.-M., Besch Williford, C., Lubahn, D.B., vom Saal, F.S., 2002. Imprinting of uterine response to estradiol and ribosomal gene methylation due to fetal exposure to diethylstilbestrol and methoxychlor in CD-1 mice: opposite effects of low and high doses. Toxcol. Appl. Pharm. 183, 10–22.

APM (Association of Plastics Manufacturers in Europe), 2005. Hyperbole or common sense? Chem. Ind., March 7, pp. 14–15.

Arukwe, A., Celius, T., Walther, B.T., Goksoyr, A., 2000. Effects of xenoestrogen treatment on *zona radiata* protein and vitellogenin expression in Atlantic salmon (*Salmo salar*). Aquat. Toxicol. 49 (3), 159–170.

Ashby, J., Tinwell, H., Haseman, J., 1999. Lack of effects for low dose levels of bisphenol A (BPA) and diethylstilbestrol (DES) on the prostate gland of CF1 mice exposed in utero. Reg. Toxicol. Pharm. 30, 156–166.

Ashby, J., Tinwell, H., Lefevre, P.A., Joiner, R., Haseman, J., 2003. The effect on sperm production in adult Sprague–Dawley rats exposed by gavage to bisphenol A between postnatal days 91–97. Toxicol. Sci. 74 (1), 129–138.

Ashby, J., Tinwell, H., Odum, J., Lefevre, P., 2004. Natural variability and the influence of concurrent control values on the detection and interpretation of low-dose or weak endocrine toxicities. Environ. Health Perspect. 112 (8), 847–853.

Barnes, D.E., Bero, L.A., 1996. Industry-funded research and conflict of interest: an analysis of research sponsored by the tobacco industry through the Center for Indoor Air Research. J. Health Polit. Policy Law 21 (3), 515–542.

Berg, C., Halldin, K., Brunstrom, B., 2001. Effects of bisphenol A and tetrabromobisphenol A on sex organ development in quail and chicken embryos. Environ. Toxicol. Chem. 20, 2836–2840.

Bisphenol-A-Global-Industry-Group, 2003. Bisphenol A and the low dose issue, http://www.bisphenol-a.org. June 20, 2003.

Burridge, E., 2003. Bisphenol A: product profile. Eur. Chem. News, April 14–20, p. 17.

Cagen, S.Z., Waechter, J.M., Dimond, S.S., Breslin, W.J., Butala, J.H., Jekat, F.W., Joiner, R.L., Shiotsuka, R.N., Veenstra, G.E., Harris, L.R., 1999. Normal reproductive organ development in CF-1 mice following prenatal exposure to bisphenol A. Toxcol. Sci. 11, 15–29.

Calabrese, E.J., Baldwin, L.A., 1997. The dose determines the stimulationa (and poison): development of a chemical hormesis database. Int. J. Toxicol. 16, 545–559.

Canesi, L., Betti, M., Lorusso, L.C., Ciacci, C., Gallo, G., 2005. 'In vivo' effects of bisphenol A in *Mytilus* hemocytes: modulation of kinase-mediated signalling pathways. Aquat. Toxicol. 71 (1), 73–84.

Carey, F.A., 2003. Organic Chemistry. McGraw-Hill, Boston.

Carr, R., Bertasi, F., Betancourt, A., Bowers, S., Gandy, B.S., Ryan, P., Willard, S., 2003. Effect of neonatal rat bisphenol A exposure on performance in the Morris water maze. J. Toxicol. Environ. Health A 66 (21), 2077–2088.

Chitra, K.C., Latchoumycandane, C., Mathur, P.P., 2003. Induction of oxidative stress by bisphenol A in the epididymal sperm of rats. Toxicology 185 (1–2), 119–127.

Colerangle, J.B., Roy, D., 1997. Profound effects of the weak environmental estrogen-like chemical bisphenol A on the growth of the mammary gland of Noble rats. J. Steroid Biochem. Mol. Biol. 60 (1–2), 153–160.

Coors, A., Jones, P.D., Giesy, J.P., Ratte, H.T., 2003. Removal of estrogenic activity from municipal waste landfill leachate assessed with a bioassay based on reporter gene expression. Environ. Sci. Technol. 37 (15), 3430–3434.

Coser, K.R., Chesnes, J., Hur, J., Ray, S., Isselbacher, K.J., Shioda, T., 2003. Global analysis of ligand sensitivity of estrogen inducible and suppressible genes in MCF7/BUS breast cancer cells by DNA microarray. Proc. Natl. Acad. Sci. USA 100, 13994–13999.

Darmani, H., Al-Hiyasat, A.S., 2004. Reproductive toxic effect of bisphenol A dimethacrylate in mice. J. Biomed. Mater. Res. 69A, 637–643.

Della Seta, D., Minder, I., Dessi-Fulgheri, F., Farabollini, F., 2005. Bisphenol-A exposure during pregnancy and lactation affects maternal behavior in rats. Brain Res. Bull. 65 (3), 255–260.

Dessi-Fulgheri, F., Porrini, S., Farabollini, F., 2002. Effects of perinatal exposure to bisphenol A on play behavior of female and male juvenile rats. Environ. Health Perspect. 110 (Suppl. 3), 403–407.

Dodds, E.C., Lawson, W., 1936. Synthetic oestrogenic agents without the phenanthrene nucleus. Nature 137, 996.

Dodds, E.C., Lawson, W., Noble, R.L., 1938. Biological effects of the synthetic oestrogenic substance 4: 4′-dihydroxy- a: B-dimethylstilbene. Lancet 234, 1389–1391.

Duft, M., Schulte-Oehlmann, U., Weltje, L., Tillmann, M., Oehlmann, J., 2003. Stimulated embryo production as a parameter of estrogenic exposure via sediments in the freshwater mudsnail *Potamopyrgus antipodarum*. Aquat. Toxicol. 64 (4), 437–449.

ECB (European Chemicals Bureau), 2003. Bisphenol A: European Union Risk Assessment Report (CAS No: 80-05-7), vol. 37. Office for Official Publications of the European Communities, Luxembourg.

Elswick, B.A., Welsch, F., Janszen, D.B., 2000. Effect of different sampling designs on outcome of endocrine disruptor studies. Reprod. Toxicol. 14, 359–367.

Ema, M., Fujii, S., Furukawa, M., Kiguchi, M., Ikka Tand Harazono, A., 2001. Rat two-generation reproductive toxicity study of bisphenol A. Reprod. Toxicol. 15, 505–523.

EPA, 2002. EPA Statement Regarding Endocrine Disruptor Low-Dose Hypothesis. United States Environmental Protection Agency, Washington, DC.

Evans, N.P., North, T., Dye, S., Sweeney, T., 2004. Differential effects of the endocrine-disrupting compounds bisphenol-A and octylphenol on gonadotropin secretion, in prepubertal ewe lambs. Domest. Anim. Endocrinol. 26 (1), 61–73.

Facciolo, R.M., Alo, R., Madeo, M., Canonaco, M., Dessi-Fulgheri, F., 2002. Early cerebral activities of the environmental estrogen bisphenol A appear to act via the somatostatin receptor subtype sst_2. Environ. Health Perspect. 110 (Suppl. 3), 397–402.

Factor, A., 1996. Mechanisms of thermal and photodegradations of bisphenol A polycarbonate. In: Clough, R.L., Billingham, N.C., Gillen, K.T. (Eds.), Polymer Durability: Degradation, Stabilization, and Lifetime Prediction. American Chemistry Society, Washington, DC, pp. 59–76.

Farabollini, F., Porrini, S., Dessi-Fulgheri, F., 1999. Perinatal exposure to the estrogenic pollutant bisphenol A affects behavior in male and female rats. Pharmacol. Biochem. Behav. 64, 687–694.

Farabollini, F., Porrini, S., Della Seta, D., Bianchi, F., Dessi-Fulgheri, F., 2002. Effects of perinatal exposure to bisphenol A on sociosexual behavior of female and male rats. Environ. Health Perspect. 110 (Suppl. 3), 409–414.

Fisher, J.S., Turner, K.J., Brown, D., Sharpe, R.M., 1999. Effect of neonatal exposure to estrogenic compounds on development of the excurrent ducts of the rat testis through puberty to adulthood. Environ. Health Perspect. 107 (5), 397–405.

Funabashi, T., Kawaguchi, M., Kimura, F., 2001. The endocrine disrupters butyl benzyl phthalate and bisphenol A increase the expression of progesterone receptor messenger ribonucleic acid in the preoptic area of adult ovariectomized rats. Neuroendocrinology 74, 77–81.

Funabashi, T., Sano, A., Mitsushima, D., Kimura, F., 2003. Bisphenol A increases progesterone receptor immunoreactivity in the hypothalamus in a dose-dependent manner and affects sexual behaviour in adult ovariectomized rats. J. Neuroendocrinol. 15 (2), 134–140.

Funabashi, T., Nakamura, T.J., Kimura, F., 2004. *p*-Nonylphenol, 4-tert-octylphenol and bisphenol A increase the expression of progesterone receptor mRNA in the frontal cortex of adult ovariectomized rats. J. Neuroendocrinol. 16 (2), 99–104.

Goloubkova, T., Ribeiro, M.F., Rodrigues, L.P., Cecconello, A.L., Spritzer, P.M., 2000. Effects of xenoestrogen bisphenol A on uterine and pituitary weight, serum prolactin levels and immunoreactive prolactin cells in ovariectomized Wistar rats. Arch. Toxicol. 74, 92–98.

Gould, J.C., Leonard, L.S., Maness, S.C., Wagner, B.L., Conner, K., Zacharewski, T., Safe, S., McDonnell, D.P., Gaido, K.W., 1998. Bisphenol A interacts with the estrogen receptor alpha in a distinct manner from estradiol. Mol. Cell. Endocrinol. 142, 203–214.

Gray, G.M., Cohen, J.T., Cunha, G., Hughes, C., McConnell, E.E., Rhomberg, L., Sipes, I.G., Mattison, D., 2004. Weight of the evidence evaluation of low-dose reproductive and developmental effects of bisphenol A. Hum. Ecol. Risk Assess. 10, 875–921.

Gupta, C., 2000a. Reproductive malformation of the male offspring following maternal exposure to estrogenic chemicals. Proc. Soc. Exp. Biol. Med. 224, 61–68.

Gupta, C., 2000b. The role of estrogen receptor, androgen receptor and growth factors in diethylstilbestrol-induced programming of prostate differentiation. Urol. Res. 28, 223–229.

Hahn, T., Schenk, K., Schulz, R., 2002. Environmental chemicals with known endocrine potential affect yolk protein content in the aquatic insect *Chironomus riparius*. Environ. Pollut. 120 (3), 525–528.

Haubruge, E., Petit, F., Gage, M.J.G., 2000. Reduced sperm counts in guppies (*Poecilia reticulata*) following exposure to low levels of tributyltin and bisphenol A. Proc. R. Soc. London 267, 2333–2337.

Honkanen, J.O., Holopainen, I.J., Kukkonen, J.V., 2004. Bisphenol A induces yolk-sac oedema and other adverse effects in landlocked salmon (*Salmo salar* m. sebago) yolk-sac fry. Chemosphere 55 (2), 187–196.

Honma, S., Suzuki, A., Buchanan, D.L., Katsu, Y., Watanabe, H., Iguchi, T., 2002. Low dose effect of in utero exposure to bisphenol A and diethylstilbestrol on female mouse reproduction. Reprod. Toxicol. 16, 117–122.

Howdeshell, K.L., Hotchkiss, A.K., Thayer, K.A., Vandenbergh, J.G., vom Saal, F.S., 1999. Exposure to bisphenol A advances puberty. Nature 401, 763–764.

Howdeshell, K.L., Peterman, P.H., Judy, B.M., Taylor, J.A., Orazio, C.E., Ruhlen, R.L., vom Saal, F.S., Welshons, W.V., 2003. Bisphenol A is released from used polycarbonate animal cages into water at room temperature. Environ. Health Perspect. 111, 1180–1187.

Hunt, P.A., Koehler, K.E., Susiarjo, M., Hodges, C.A., Hagan, A., Voigt, R.C., Thomas, S., Thomas, B.F., Hassold, T.J., 2003. Bisphenol A causes meiotic aneuploidy in the female mouse. Curr. Biol. 13, 546–553.

Imanishi, S., Manabe, N., Nishizawa, H., Morita, M., Sugimoto, M., Iwahori, M., Miyamoto, H., 2003. Effects of oral exposure of bisphenol A on mRNA expression of nuclear receptors in murine placentae assessed by DNA microarray. J. Reprod. Dev. 49, 329–336.

IRIS, 1988. Bisphenol A (CASRN 80-05-7), US-EPA Integrated Risk Information System Substance file. http://www.epa.gov/iris/subst/0356.htm. Access date: January 2, 2005.

Ishido, M., Masuo, Y., Kunimoto, M., Oka, S., Morita, M., 2004. Bisphenol A causes hyperactivity in the rat concomitantly with

impairment of tyrosine hydroxylase immunoreactivity. J. Neurosci. Res. 76 (3), 423–433.
Ishihara, A., Nishiyama, N., Sugiyama, S., Yamauchi, K., 2003. The effect of endocrine disrupting chemicals on thyroid hormone binding to Japanese quail transthyretin and thyroid hormone receptor. Gen. Comp. Endocrinol. 134 (1), 36–43.
Jefferson, W.N., Padilla-Banks, E., Newbold, R.R., 2005. Adverse effects on female development and reproduction in CD-1 mice following neonatal exposure to the phytoestrogen genistein at environmentally relevant doses. Biol. Reprod., Online, June 1.
Jobling, S., Casey, D., Rodgers-Gray, T., Oehlmann, J., Schulte-Oehlmann, U., Pawlowski, S., Baunbeck, T., Turner, A.P., Tyler, C.R., 2003. Comparative responses of molluscs and fish to environmental estrogens and an estrogenic effluent. Aquat. Toxicol. 65 (2), 205–220.
Kabuto, H., Hasuike, S., Minagawa, N., Shishibori, T., 2003. Effects of bisphenol A on the metabolisms of active oxygen species in mouse tissues. Environ. Res. 93 (1), 31–35.
Kabuto, H., Amakawa, M., Shishibori, T., 2004. Exposure to bisphenol A during embryonic/fetal life and infancy increases oxidative injury and causes underdevelopment of the brain and testis in mice. Life Sci. 74 (24), 2931–2940.
Kashiwada, S., Ishikawa, H., Miyamoto, N., Ohnishi, Y., Magara, Y., 2002. Fish test for endocrine-disruption and estimation of water quality of Japanese rivers. Water Res. 36 (8), 2161–2166.
Kawagoshi, Y., Fujita, Y., Kishi, I., Fukunaga, I., 2003. Estrogenic chemicals and estrogenic activity in leachate from municipal waste landfill determined by yeast two-hybrid assay. J. Environ. Monit. 5 (2), 269–274.
Kawai, K., Takehiro, N., Nishikata, H., Aou, S., Takii, M., Kubo, C., 2003. Aggressive behavior and serum testosterone concentration during the maturation process of male mice: the effects of fetal exposure to bisphenol A. Environ. Health Perspect. 111, 175–178.
Khurana, S., Ranmal, S., Ben-Jonathan, N., 2000. Exposure of newborn male and female rats to environmental estrogens: delayed and sustained hyperprolactinemia and alterations in estrogen receptor expression. Endocrinology 141, 4512–4517.
Kloas, W., Lutz, I., Einspanier, R., 1999. Amphibians as a model to study endocrine disruptors: II. Estrogenic activity of environmental chemicals in vitro and in vivo. Sci. Total Environ. 225, 59–68.
Kubo, K., Arai, O., Ogata, R., Omura, M., Hori, T., Aou, S., 2001. Exposure to bisphenol A during the fetal and suckling periods disrupts sexual differentiation of the locus coeruleus and of behaviour in the rat. Neurosci. Lett. 304, 73–76.
Kubo, K., Arai, O., Omura, M., Watanabe, R., Ogata, R., Aou, S., 2003. Low dose effects of bisphenol A on sexual differentiation of the brain and behavior in rats. Neurosci. Res. 45 (3), 345–356.
Kwak, H.I., Bae, M.O., Lee, M.H., Lee, Y.S., Lee, B.J., Kang, K.S., Chae, C.H., Sung, H.J., Shin, J.S., Kim, J.H., Mar, W.C., Sheen, Y.Y., Cho, M.H., 2001. Effects of nonylphenol, bisphenol A, and their mixture on the viviparous swordtail fish (*Xiphophorus helleri*). Environ. Toxicol. Chem. 20 (4), 787–795.
Laviolaa, G., Gioiosa, L., Adriania, W., Palanza, P., 2005. D-amphetamine-related reinforcing effects are reduced in mice exposed prenatally to estrogenic endocrine disruptors. Brain Res. Bull. 65, 235–240.
Lee, H.J., Chattopadhyay, S., Gong, E.Y., Ahn, R.S., Lee, K., 2003. Antiandrogenic effects of bisphenol A and nonylphenol on the function of androgen receptor. Toxicol. Sci. 75 (1), 40–46.
Lemmen, J.G., Arends, R.J., van der Saag, P.T., van der Burg, B., 2004. In vivo imaging of activated estrogen receptors in utero by estrogens and bisphenol A. Environ. Health Perspect. 112 (15), 1544–1549.
Levy, G., Lutz, I., Kruger, A., Kloas, W., 2004. Bisphenol A induces feminization in *Xenopus laevis* tadpoles. Environ. Res. 94 (1), 102–111.
Lindholst, C., Pedersen, K.L., Pedersen, S.N., 2000. Estrogenic response of bisphenol A in rainbow trout (*Oncorhynchus mykiss*). Aquat. Toxicol. 48 (2–3), 87–94.
Long, X., Steinmetz, R., Ben-Jonathan, N., caperell-Grant, A., Young, P.C.M., Nephew, K.P., Bigsby, R.M., 2000. Strain differences in vaginal responses to the xenoestrogen bisphenol A. Environ. Health Perspect. 108, 243–247.
MacLusky, N.J., Hajszan, T., Leranth, C., 2005. The environmental estrogen bisphenol A inhibits estrogen-induced hippocampal synaptogenesis. Environ. Health Perspect. 113, 675–679.
Markey, C.M., Luque, E.H., Munoz De Toro, M., Sonnenschein, C., Soto, A.M., 2001a. In utero exposure to bisphenol A alters the development and tissue organization of the mouse mammary gland. Biol. Reprod. (See erratum in Biol. Reprod. 71(5): 1753, 2004, stating that the doses were 25 and 250 ng/kg/day).
Markey, C.M., Michaelson, C.L., Veson, E.C., Sonnenschein, C., Soto, A.M., 2001b. The mouse uterotrophic assay: a reevaluation of its validity in assessing the estrogenicity of bisphenol A. Environ. Health Perspect. 109 (1), 55–60.
Markey, C.M., Coombs, M.A., Sonnenschein, C., Soto, A.M., 2003. Mammalian development in a changing environment: exposure to endocrine disruptors reveals the developmental plasticity of steroid-hormone target organs. Evol. Dev. 5 (1), 67–75.
Markey, C.M., Wadia, P.R., Rubin, B.S., Sonnenschein, C., Soto, A.M., 2005. Long-term effects of fetal exposure to low doses of the xenoestrogen bisphenol-A in the female mouse genital tract. Biol. Reprod. 72 (6), 1344–1351.
Markowitz, G., Rosner, D., 2000. "Cater to the children": the role of the lead industry in a public health tragedy, 1900–1955. Am. J. Public Health 90, 36–46.
Masuo, Y., Ishido, M., Morita, M., Oka, S., 2004. Effects of neonatal treatment with 6-hydroxydopamine and endocrine disruptors on motor activity and gene expression in rats. Neural Plast. 11 (1–2), 59–76.
Metcalfe, C.D., Metcalfe, T.L., Kiparissis, Y., Koenig, B.G., Khan, C., Hughes, R.J., Croley, T.R., March, R.E., Potter, T., 2001. Estrogenic potency of chemicals detected in sewage treatment plant effluents as determined by *in vivo* assays with japanese medaka (*Oryzias latipes*). Environ. Toxicol. Chem. 20 (2), 297–308.
Michaels, D., 2005. Doubt is their product. Sci. Am. 292, 96–101.
Michaels, D., Monforton, C., 2005. Manufacturing uncertainty: Contested science and the protection of the public's health and environment. Am. J. Public Health 95 (Suppl 1), S39–S48.
Milloy, S., 2005. California's bogus baby bottle scare. July 16 issue, http://www.junkscience.com. July 16, 2005.
Montano, M.M., Wang, M., Even, M.D., vom Saal, F.S., 1991. Serum corticosterone in fetal mice: sex differences, circadian changes, and effect of maternal stress. Physiol. Behav. 50, 323–329.
Montano, M.M., Welshons, W.V., vom Saal, F.S., 1995. Free estradiol in serum and brain uptake of estradiol during fetal and neonatal sexual differentiation in female rats. Biol. Reprod. 53 (5), 1198–1207.
Moriyama, K., Tagami, T., Akamizu, T., Usui, T., Saljo, M., Kanamoto, N., Hataya, Y., Shimatsu, A., Kuzuya, H., Nakao, K., 2002. Thyroid hormone action is disrupted by bisphenol A as an antagonist. J. Clin. Endocrinol. Metab. 87, 5185–5190.
Morrissey, R.E., George, J.D., Price, C.J., Tyl, R.W., Marr, M.C., Kimmel, C.A., 1987. The developmental toxicity of bisphenol A in rats and mice. Fund. Appl. Toxicol. 8, 571–582.
Munoz-de-Toro, M., Markey, C., Wadia, P. R., Luque, E. H., Rubin, B. S., A, S.C. Soto, M., 2005. Perinatal exposure to bisphenol A alters peripubertal mammary gland development in mice. Endocrinology, Online: May 31.
Nagel, S.C., vom Saal, F.S., Thayer, K.A., Dhar, M.G., Boechler, M., Welshons, W.V., 1997. Relative binding affinity-serum modified access (RBA-SMA) assay predicts the relative in vivo bioactivity of the xenoestrogens bisphenol A and octylphenol. Environ. Health Perspect. 105 (1), 70–76.
Nagel, S.C., Hagelbarger, J.L., McDonnell, D.P., 2001. Development of an ER action indicator mouse for the study of estrogens, selective ER modulators (SERMs), and xenobiotics. Endocrinology 142 (11), 4721–4728.

Needleman, H.L., Epstein, S., Carnow, B., Scanlon, J., Parkinson, D., Samuels, S., Mazzzochi, A., David, O., 1975. Blood-lead levels, behaviour, and intelligence. Lancet (March 29), 751.

Negishi, T., Kawasaki, K., Takatori, A., Ishii, Y., Kyuwa, S., Kuroda, Y., Yoshikawa, Y., 2003. Effects of perinatal exposure to bisphenol A on the behavior of offspring in F344 rats. Environ. Toxicol. Pharmacol. 14 (3), 99–108.

Negishi, T., Tominaga, T., Ishii, Y., Kyuwa, S., Hayasaka, I., Kuroda, Y., Yoshikawa, Y., 2004. Comparative study on toxicokinetics of bisphenol A in F344 rats, monkeys (*Macaca fascicularis*), and chimpanzees (*Pan troglodytes*). Exp. Anim. 53(4), 391–394.

Newbold, R.R., Banks, E.P., Bullock, B., Jefferson, W.N., 2001. Uterine adenocarcinoma in mice treated neonatally with genistein. Cancer Res. 61, 4325–4328.

Newbold, R.R., Jefferson, W.N., Padilla-Banks, E., Haseman, J., 2004. Developmental exposure to diethylstilbestrol (DES) alters uterine response to estrogens in prepubescent mice: low versus high dose effects. Reprod. Toxicol. 18, 399–406.

Newbold, R.R., Padilla-Banks, E., Snyder, R.J., Jefferson, W.N., 2005. Developmental exposure to estrogenic compounds and obesity. Birth Defects Res. A: Clin. Mol. Teratol. 73 (7), 478–480.

Nikaido, Y., Yoshizawa, K., Danbara, N., Tsujita-Kyutoku, M., Yuri, T., Uehara, N., Tsubura, A., 2004. Effects of maternal xenoestrogen exposure on development of the reproductive tract and mammary gland in female CD-1 mouse offspring. Reprod. Toxicol. 18 (6), 803–811.

Nishizawa, H., Manabe, N., Morita, M., Sugimoto, M., Imanishi, S., Miyamoto, H., 2003. Effects of in utero exposure to bisphenol A on expression of RARalpha and RXRalpha mRNAs in murine embryos. J. Reprod. Dev. 49 (6), 539–545.

Nishizawa, H., Morita, M., Sugimoto, M., Imanishi, S., Manabe, N., 2005. Effects of in utero exposure to bisphenol A on mRNA expression of arylhydrocarbon and retinoid receptors in murine embryos. J. Reprod. Dev. 51 (3), 315–324.

Nonneman, D.J., Ganjam, V.K., Welshons, W.V., vom Saal, F.S., 1992. Intrauterine position effects on steroid metabolism and steroid receptors of reproductive organs in male mice. Biol. Reprod. 47, 723–729.

NTP, 2001. Final report of the endocrine disruptors low dose peer review panel, http://ntp-server.niehs.nih.gov/index.cfm?objectid=06F5CE98-E82F-8182-7FA81C02D3690D47. August 20, 2005.

Nunez, A.A., Kannan, K., Giesy, J.P., Fang, J., Clemens, L.G., 2001. Effects of bisphenol A on energy balance and accumulation in brown adipose tissue in rats. Chemosphere 42, 917–922.

Oehlmann, J., Schulte-Oehlmann, U., Tillmann, M., Markert, B., 2000. Effects of endocrine disruptors on prosobranch snails (Mollusca: Gastropoda) in the laboratory. Part I: bisphenol A and octylphenol as xeno-estrogens. Ecotoxicology 9 (6), 383–397.

Ohsako, S., Tohyama, C., 2005. Comparison of study controls. Environ. Health Perspect. 113, A582.

OMB_Watch, 2005. Fetal harm, culture of life, and unsound science. May 5 edition, http://www.ombwatch.org/article/blogs/279/0/2005/5. August 10, 2005.

Ong, E.K., Glantz, S.A., 2001. Constructing "sound science"' and "good epidemiology": Tobacco, lawyers, and public relations firms. Am. J. Public Health 91, 1749–1756.

Palanza, P., Howdeshell, K.L., Parmigiani, S., vom Saal, F.S., 2002. Exposure to a low dose of bisphenol A during fetal life or in adulthood alters maternal behavior in mice. Environ. Health Perspect. 110, 415–422.

Papaconstantinou, A.D., Fisher, B.R., Umbreit, T.H., Brown, K.M., 2002. Increases in mouse uterine heat shock protein levels are a sensitive and specific response to uterotrophic agents. Environ. Health Perspect. 110 (12), 1207–1212.

Paris, F., Balaguer, P., Terouanne, B., Servant, N., Lacoste, C., Cravedi, J.P., Nicolas, J.C., Sultan, C., 2002. Phenylphenols, biphenols, bisphenol-A and 4-tert-octylphenol exhibit alpha and beta estrogen activities and antiandrogen activity in reporter cell lines. Mol. Cell Endocrinol. 193 (1–2), 43–49.

Pearson, H., 2003. Plastics spoil mouse eggs. April 1 edition, http://www.nature.com/nsu/030331/030331-2.html. August 20, 2005.

Porrini, S., Belloni, V., Seta, D.D., Farabollini, F., Giannelli, G., Dessi-Fulgheri, F., 2005. Early exposure to a low dose of bisphenol A affects socio-sexual behavior of juvenile female rats. Brain Res. Bull. 65 (3), 261–266.

Purchase, I.F.H., 2004. Fraud, errors and gamesmanship in experimental toxicology. Toxicology 202, 1–20.

Putz, O., Schwartz, C.B., Kim, S., LeBlanc, G.A., Cooper, R.L., Prins, G.S., 2001. Neonatal low- and high-dose exposure to estradiol benzoate in the male rat: 1. Effects on the prostate gland. Biol. Reprod. 65, 1496–1505.

Quesada, I., Fuentes, E., Viso-Leon, M.C., Soria, B., Ripoll, C., Nadal, A., 2002. Low doses of the endocrine disruptor bisphenol-A and the native hormone 17beta-estradiol rapidly activate transcription factor CREB. FASEB J. 16 (12), 1671–1673.

Ramos, J.G., Varayoud, J., Sonnenschein, C., Soto, A.M., Munoz De Toro, M., Luque, E.H., 2001. Prenatal exposure to low doses of bisphenol A alters the periductal stroma and glandular cell function in the rat ventral prostate. Biol. Reprod. 65 (4), 1271–1277.

Ramos, J.G., Varayoud, J., Kass, L., Rodriguez, H., Costabel, L., Munoz-De-Toro, M., Luque, E.H., 2003. Bisphenol A induces both transient and permanent histofunctional alterations of the hypothalamic–pituitary–gonadal axis in prenatally exposed male rats. Endocrinology 144 (7), 3206–3215.

Razzoli, M., Valsecchi, P., Palanza, P., 2005. Chronic exposure to low doses bisphenol A interferes with pair-bonding and exploration in female Mongolian gerbils. Brain Res. Bull. 65 (3), 249–254.

Rosenthal, R., Rosnow, R.L., 1991. Essentials of Behavioral Research: Methods and Data Analysis. NcGraw-Hill, New York.

Rubin, B.S., Murray, M.K., Bamassa, D.A., King, J.C., Soto, A.M., 2001. Perinatal exposure to low doses of bisphenol A affects body weight, patterns of estrous cyclicity, and plasma LH levels. Environ. Health Perspect. 109, 657–680.

Sakaue, M., Ohsako, S., Ishimura, R., Kurosawa, S., Kurohmaru, M., Hayashi, Y., Aoki, Y., Yonemoto, J., Tohyama, C., 2001. Bisphenol A affects spermatogenesis in the adult rat even at a low dose. J. Occup. Health 43, 185–190.

Sawai, C., Anderson, K., Walser-Kuntz, D., 2003. Effect of bisphenol A on murine immune function: modificattion of interferon-gamma, IgG2a, and disease symptoms in NZB × NZW F1 mice. Environ. Health Perspect. 111 (16), 1883–1887.

Schönfelder, G., Wittfoht, W., Hopp, H., Talsness, C.E., Paul, M., Chahoud, I., 2002a. Parent bisphenol A accumulation in human maternal–fetal–placental unit. Environ. Health Perspect. 110, A703–A707.

Schönfelder, G., Flick, B., Mayr, L., Talsness, C., Paul, M., Chahoud, I., 2002b. In utero exposure to low doses of bisphenol A lead to long-term deleterious effects in the vagina. Neoplasia 4, 98–102.

Schönfelder, G., Friedrich, K., Paul, M., Chahoud, I., 2004. Developmental effeccts of prenatal exposure to bisphenol A on the uterus of rat offspring. Neoplasia 6, 584–594.

Schulte-Oehlmann, U., Tillmann, M., Casey, D., Duft, M., Markert, B., Oehlmann, J., 2001. Xeon-estrogenic eeffects of bisphenol A in prosobranchs (Mollusca: Gaastropoda: prosbranchia). Z. Umweltchem. Okotoxicol. 13, 319–333.

ScienceNewsOnline, 1999. Food for thought: what's coming out of baby's bottle? Sci. News, Online 156: pp. 1–4, www.sciencenews.org/sn_arc99/8_7_99/food.htm. August 20, 2005.

Sheehan, D., Young, M., 1979. Diethylstilbestrol and estradiol binding to serum albumin and pregnancy plasma of rats and humans. Endocrinology 104, 1442–1446.

Sheehan, D.M., Branham, W.S., 1987. Dissociation of estrogen-induced uterine growth and ornithine decarboxylase activity in the postnatal rat. Teratogen. Carcinogen. Mutagen. 7, 411–422.

Sheehan, D.M., Willingham, E., Gaylor, D., Bergeron, J.M., Crews, D., 1999. No threshold dose for estradiol-induced sex reversal of turtle embryos: how little is too much? Environ. Health Perspect. 107 (2), 155–159.

Shioda, T., Wakabayashi, M., 2000. Effect of certain chemicals on the reproduction of medaka (*Oryzias latipes*). Chemosphere 40, 239–243.

Snyder, R.W., Maness, S.C., Guido, K.W., Welsch, F., Summer, S.C.J., Fennell, T.R., 2000. Metabolism and disposition of bisphenol A in female rats. Toxicol. Appl. Pharm. 168, 225–234.

Society-of-the-Plastics-Industry, 1995. Bisphenol A (BPA) Toxicology Task Force, Toxicology and Estrogenicity Summary. Society-of-the-Plastics-Industry, Washington, DC.

Society-of-the-Plastics-Industry, 1996. Report on the potential exposures to bisphenol A from epoxy can coatings. Society-of-the-Plastics-Industry, Washington, DC.

Sohoni, P., Tyler, C.R., Hurd, K., Caunter, J., Hetheridge, M., Williams, T., Woods, C., Evans, M., Toy, R., Gargas, M., Sumpter, J.P., 2001. Reproductive effects of long-term exposure to bisphenol A in the fathead minnow (*Pimephales promelas*). Environ. Sci. Technol. 35 (14), 2917–2925.

Steinmetz, R., Brown, N.G., Allen, D.L., Bigsby, R.M., Ben-Jonathan, N., 1997. The environmental estrogen bisphenol A stimulates prolactin release in vitro and in vivo. Endocrinology 138 (5), 1780–1786.

Steinmetz, R., Mitchner, N.A., Grant, A., Allen, D.L., Bigsby, R.M., Ben-Jonathan, N., 1998. The xenoestrogen bisphenol A induces growth, differentiation, and c-fos gene expression in the female reproductive tract. Endocrinology 139, 2741–2747.

Sugita-Konishi, Y., Shimura, S., Nishikawa, T., Sunaga, F., Naito, H., Suzuki, Y., 2003. Effect of Bisphenol A on non-specific immunodefenses against non-pathogenic *Escherichia coli*. Toxicol. Lett. 136 (3), 217–227.

Sugiura-Ogasawara, M., Ozaki, Y., Sonta, S., Makino, T., Suzumori, K., 2005. Exposure to bisphenol A is associated with recurrent miscarriage. Hum. Reprod. 20, 2325–2329.

Suzuki, A., Sugihara, A., Uchida, K., Sato, T., Ohta, Y., Katsu, Y., Watanabe, H., Iguchi, T., 2002. Developmental effects of perinatal exposure to bisphenol A and diethylstilbestrol on reproductive organs in female mice. Reprod. Toxicol. 16, 107–116.

Suzuki, T., Mizuo, K., Nakazawa, H., Funae, Y., Fushiki, S., Fukushima, S., Shirai, T., Narita, M., 2003. Prenatal and neonatal exposure to bisphenol-A enhances the central dopamine D1 receptor-mediated action in mice: enhancement of the methamphetamine-induced abuse state. Neuroscience 117 (3), 639–644.

Tabata, A., Kashiwada, S., Ohnishi, Y., Ishikawa, H., Miyamoto, N., Itoh, M., Magara, Y., 2001. Estrogenic influences of estradiol-17β, *o*-nonylphenol, and bisphenol A on Japanese medaka (*Oryzias latipes*) at detected environmental concentrations. Water Sci. Technol. 43, 109–116.

Tabata, A., Miyamoto, N., Ohnishi, Y., Itoh, M., Yamada, T., Kamei, T., Magara, Y., 2003. The effect of chlorination of estrogenic chemicals on the level of serum vitellogenin of Japanese medaka (*Oryzias latipes*). Water Sci. Technol. 47 (9), 51–57.

Takagi, H., Shibutani, M., Masutomi, N., Uneyama, C., Takahashi, N., Mitsumori, K., Hirose, M., 2004. Lack of maternal dietary exposure effects of bisphenol A and nonylphenol during the critical period for brain sexual differentiation on the reproductive/endocrine systems in later life. Arch. Toxicol. 78(2), 97–105.

Takahashi, O., Oishi, S., 2003. Testicular toxicity of dietarily or parenterally administered bisphenol A in rats and mice. Food Chem. Toxicol. 41 (7), 1035–1044.

Takao, Y., Chul Lee, H., Ishibashi, Y., Kohra, S., Tominaga, N., Arizono, K., 1999a. Fast screening method for bisphenol A in environmental water and in food by solid-phase microextraction (SPME). J. Health Sci. 45, 39.

Takao, T., Nanamiya, W., Nagano, I., Asaba, K., Kawabata, K., Hashimoto, K., 1999b. Exposure with the environmental estrogen bisphenol A disrupts the male reproductive tract in young mice. Life Sci. 65(22), 2351–2357.

Takao, T., Nanamiya, W., Nazarloo, H.P., Matsumoto, R., Asaba, K., Hashimoto, K., 2003. Exposure to the environmental estrogen bisphenol A modulated estrogen receptor-alpha and -beta immunoreactivity and mRNA in male mouse testis. Life Sci. 10, 1159–1169.

Takeuchi, T., Tsutsumi, O., Ikezuki, Y., Takai, Y., Taketani, Y., 2004. Positive relationship between androgen and the endocrine disruptor, bisphenol A, in normal women and women with ovarian dysfunction. Endocr. J. 51 (2), 165–169.

Talsness, C., Fialkowski, O., Gericke, C., Merker, H.-J., Chahoud, I., 2000. The effects of low and high doses of bisphenol A on the reproductive system of female and male rat offspring. Congenit. Anomal. 40, S94–S107.

Tang, W.-Y., Belmonte, J., Prins, G.S., Ho, S.-M., 2005. Discovery of Phosphodiesterase Type 4 Variant (PDE-4D4) as a Gene Susceptible to Neonatal Imprinting by Estradiol or Bisphenol A in the Rat Prostate. The Forum on Endocrine Disrupting Chemicals, The Endocrine Society, San Diego.

Teeguarden, J.G., Waechter Jr., J.M., Clewell III, H.J., Covington, T.R., Barton, H.A., 2005. Evaluation of oral and intravenous route pharmacokinetics, plasma protein binding, and uterine tissue dose metrics of bisphenol A: a physiologically based pharmacokinetic approach. Toxicol. Sci. 85 (2), 823–838.

Thayer, K.A., Ruhlen, R.L., Howdeshell, K.L., Buchanan, D., Cooke, P.S., Welshons, W.V., vom Saal, F.S., 2001. Altered reproductive organs in male mice exposed prenatally to sub-clinical doses of 17a-ethinylestradiol. Hum. Reprod. 16, 988–996.

Thigpen, J.E., Haseman, J.K., Saunders, H.E., Setchell, K.D.R., Grant, M.G., Forsythe, D.B., 2003. Dietary phytoestrogens accelerate the time of vaginal opening in immature CD-1 mice. Comp. Med. 53, 477–485.

Thuillier, R., Wang, Y., Culty, M., 2003. Prenatal exposure to estrogenic compounds alters the expression pattern of platelet-derived growth factor receptors alpha and beta in neonatal rat testis: identification of gonocytes as targets of estrogen exposure. Biol. Reprod. 68 (3), 867–880.

Timms, B.G., Howdeshell, K.L., Barton, L., Bradley, S., Richter, C.A., vom Saal, F.S., 2005. Estrogenic chemicals in plastic and oral contraceptives disrupt development of the mouse prostate and urethra. Proc. Natl. Acad. Sci. USA 102, 7014–7019.

Tohei, A., Suda, S., Taya, K., Hashimoto, T., Kogo, H., 2001. Bisphenol A inhibits testicular functions and increases luteinizing hormone secretion in adult male rats. Exp. Biol. Med. 226, 216–221.

Toloken, S., 1998. SPI study disputes endocrine disruptor findings. Plastic News, October 16.

Tong, E.K., England, L.E., Glantz, S.A., 2005. Changing conclusions on secondhand smoke in a sudden infant death syndrome review funded by the tobacco industry. Pediatrics 115 (3), 356–366.

Toyama, Y., Yuasa, S., 2004. Effects of neonatal administration of 17beta-estradiol, beta-estradiol 3-benzoate, or bisphenol A on mouse and rat spermatogenesis. Reprod. Toxicol. 19 (2), 181–188.

Trudeau, V.L., Turque, N., Le Mevel, S., Allio t, C., Gallant, N., Coen, L., Pakdel, F., Demeneix, B., 2005. Assessment of estrogenic endocrine-disrupting chemical actions in the brain using in vivo somatic gene transfer. Environ. Health Perspect. 113 (3), 329–334.

Tyl, R., Myers, C., Marr, M., Thomas, B., Keimowitz, A., Brine, D., Veselica, M., Fail, P., Chang, T., Seely, J., Joiner, R., Butala, J., Dimond, S., Cagen, S., Shiotsuka, R., Stropp, G., Waechter, J., 2002. Three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague–Dawley rats. Toxicol. Sci. 68, 121–146.

Tyl, R.W., 2003. Bisphenol A: findings of a multigenerational rat study. Environ. Health Perspect. 111 (12), A632.

Van den Belt, K., Verheyen, R., Witters, H., 2003. Comparison of vitellogenin responses in zebrafish and rainbow trout following exposure to environmental estrogens. Ecotoxicol. Environ. Saf. 56(2), 271–281.

vom Saal, F.S., 2005a. Bisphenol A: list of published articles, http://rcp.missouri.edu/endocrinedisruptors/vomsaal/vomsaal.html

vom Saal, F.S., 2005b. Low-dose BPA: confirmed by extensive literature. Chem. Ind. 7, 14–15.

vom Saal, F.S., Hughes, C., 2005. An extensive new literature concerning low-dose effects of bisphenol A shows the need for a new risk assessment. Environ. Health Perspect. 113, 926–933.

vom Saal, F.S., Sheehan, D.M., 1998. Challenging risk assessment. Forum Appl. Res. Public Pol. 13, 11–18.

vom Saal, F.S., Timms, B.G., Montano, M.M., Palanza, P., Thayer, K.A., Nagel, S.C., Dhar, M.D., Ganjam, V.K., Parmigiani, S., Welshons, W.V., 1997. Prostate enlargement in mice due to fetal exposure to low doses of estradiol or diethylstilbestrol and opposite effects at high doses. Proc. Natl. Acad. Sci. USA 94 (5), 2056–2061.

vom Saal, F.S., Cooke, P.S., Buchanan, D.L., Palanza, P., Thayer, K.A., Nagel, S.C., Parmigiani, S., Welshons, W.V., 1998. A physiologically based approach to the study of bisphenol A and other estrogenic chemicals on the size of reproductive organs, daily sperm production, and behavior. Toxicol. Ind. Health 14 (1–2), 239–260.

vom Saal, F.S., Richter, C.A., Ruhlen, R.L., Nagel, S.C., Welshons, W.V. (Eds.), 2004. Disruption of laboratory experiments due to leaching of bisphenol A from polycarbonate cages and bottles and uncontrolled variability in components of animal feed. In: Proceedings of the International Workshop on Development of Science-Based Guidelines for Laboratory Animal Care. National Academy Press, Washington, DC.

vom Saal, F.S., Nagel, S.C., Timms, B.G., Welshons, W.V., 2005. Implications for human health of the extensive bisphenol A literature showing adverse effects at low doses: a response to attempts to mislead the public. Toxicology 212, 244–252.

Wada, H., Tarumi, H., Imazato, S., Narimatsu, M., Ebisu, S., 2004. In vitro estrogenicity of resin composites. J. Dent. Res. 83 (3), 222–226.

Walsh, D.E., Dockery, P., Doolan, C.M., 2005. Estrogen receptor independent rapid non-genomic effects of environmental estrogens on $[Ca^{++}]i$ in human breast cancer cells. Mol. Cell. Endocrinol. 230 (1–2), 23–30.

Wang, H., Tranguch, S., Xie, H., Hanley, G., Das, S.K., Dey, S.K., 2005. Variation in commercial rodent diets induces disparage molecular and physiological changes in the mouse uterus. Proc. Natl. Acad. Sci. USA 102, 9960–9965.

Wang, Y., Thuillier, R., Culty, M., 2004. Prenatal estrogen exposure differentially affects estrogen receptor-associated proteins in rat testis gonocytes. Biol. Reprod. 71 (5), 1652–1664.

Watts, M.M., Pascoe, D., Carroll, K., 2001. Chronic exposure to 17alpha ethinylestradiol and bisphenol A—effects on development and reproduction in the freshwater invertebrate *Chironomus riparius* (Diptera: Chironomidae). Aquat. Toxicol. 55, 1113–1124.

Watts, M.M., Pascoe, D., Carroll, K., 2003. Exposure to 17 alpha-ethinylestradiol and bisphenol A—effects on larval moulting and mouthpart structure of Chironomus riparius. Ecotoxicol. Environ. Saf. 54 (2), 207–215.

Welshons, W.V., vom Saal, F.S., 1998. The importance pf protocol design and data reporting to research on endocrine disruption: response. Environ. Health Perspect. 106, A316–A317.

Welshons, W.V., Nagel, S.C., Thayer, K.A., Judy, B.M., vom Saal, F.S., 1999. Development of *in vitro* assays to predict activity of xenoestrogens in animals: fetal exposure to methoxychlor and other xenoestrogens increases adult prostate size in mice. Toxicol. Ind. Health 15, 12–25.

Welshons, W.V., Thayer, K.A., Judy, B.M., Taylor, J.A., Curran, E.M., vom Saal, F.S., 2003. Large effects from small exposures. I. Mechanisms for endocrine-disrupting chemicals with estrogenic activity. Environ. Health Perspect. 111, 994–1006.

Wetherill, Y.B., Petra, C.E., Monk, K.R., Puga, A., Knudsen, K.E., 2002. The xenoestrogen bisphenol A induces inappropriate androgen receptor activation and mitogenesis in prostate adenocarcinoma cells. Mol. Cancer Therapeut. 7, 515–524.

Wistuba, J., Brinkworth, M.H., Schlatt, S., Chahoud, I., Nieschlag, E., 2003. Intrauterine bisphenol A exposure leads to stimulatory effects on Sertoli cell number in rats. Environ. Res. 91 (2), 95–103.

Wozniak, A.L., Bulayeva, N.N., Watson, C.S., 2005. Xenoestrogens at picomolar to nanomolar concentrations trigger membrane estrogen receptor-a mediated Ca^{++} fluxes and prolactin release in GH3/B6 pituitary tumor cells. Environ. Health Perspect. 113, 431–439.

Yamasaki, K., Sawaki, M., Noda, S., Inmatanaka, N., Takatsuki, M., 2002. Subacute oral toxicity study of ethinylestradiol and bisphenol A, based on the draft protocol for the 'Enhanced OECD Test Guideline no. 407'. Arch. Toxicol. 76, 65–74.

Yoshino, S., Yamaki, K., Yanagisawa, R., Takano, H., Hayashi, H., Mori, Y., 2003. Effects of bisphenol A on antigen-specific antibody production, proliferative responses of lymphoid cells, and TH1 and TH2 immune responses in mice. Br. J. Pharmacol. 138 (7), 1271–1276.

Yoshino, S., Yamaki, K., Li, X., Sai, T., Yanagisawa, R., Takano, H., Taneda, S., Hayashi, H., Mori, Y., 2004. Prenatal exposure to bisphenol A up-regulates immune responses, including T helper 1 and T helper 2 responses, in mice. Immunology 112 (3), 489–495.

Zalko, D., Soto, A.M., Dolo, L., Dorio, C., Rathahao, E., Debrauwer, L., Faure, R., Cravedi, J.P., 2003. Biotransformations of bisphenol A in a mammalian model: answers and new questions raised by low-dose metabolic fate studies in pregnant CD-1 mice. Environ. Health Perspect. 111, 309–319.

Zoeller, R.T., Rovet, J., 2004. Timing of thyroid hormone action in the developing brain: clinical observations and experimental findings. J. Neuroendocrinol. 16 (10), 809–818.

Zoeller, R.T., Bansal, R., Parris, C., 2005. Bisphenol-A, an environmental contaminant that acts as a thyroid hormone receptor antagonist in vitro, increases serum thyroxine, and alters RC3/neurogranin expression in the developing rat brain. Endocrinology 146 (2), 607–612.

Zsarnovszky, A., Lee, H.H., Wang, H.-S., Belcher, S.M., 2005. Ontogeny of rapid estrogen-mediated ERK1/2 signaling in the rat cerebellar cortex in vivo: potent non-genomic agonist and endocrine disrupting activity of the xenoestrogen bisphenol A. Endocrinology, Online August 25, 2005, doi: 10.1210/en.2005-0565.